Age-related changes in neural plasticity after motor learning

Kelly Berghuis

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I Chapter 1 I

General introduction

1.1 Motor learning in aging

Motor learning occurs in stages. First, motor skill acquisition is the improvement in specific movement performance due to practice, either in temporal or spatial domain [1]. Second, during the consolidation phase in-between sessions, the motor memory is transformed from an initial fragile state to a more stable form that is resistant to interference, which can result in retention of the acquired skill or even an improvement in performance after this offline period [2, 3]. Throughout the lifespan, humans learn new motor skills and relearn motor skills after an injury. In older adults, motor learning is particularly important because adaptations to age-related peripheral and central neural changes are required [4-7]. While it has been established that older adults are able to acquire novel motor skills, such as ballistic, sequential motor, or visuomotor tracking skills [8-10], whether or not skill acquisition and consolidation are impaired in older compared with young adults is still under debate. Furthermore, it is unclear whether and how the underlying neural mechanisms of motor learning change with advancing age. This thesis focuses on unraveling these age-related changes in neural plasticity underlying motor skill acquisition after a single practice session and motor memory consolidation after 24 hours.

1.2 Neural mechanisms underlying motor learning

A main neuronal mechanism underlying motor learning is altering synaptic strength after repeated stimulation by long-term potentiation (LTP) and long-term depression (LTD), as evidenced by animal and human studies [11-13]. LTP refers to the strengthening of synapses, whereas LTD refers to the weakening of synapses. This use-dependent synaptic plasticity is influenced by glutamatergic and gamma-aminobutyric acid-ergic (GABAergic) processes. Over the past three decades, these excitatory and inhibitory processes have been measured indirectly by a non-invasive brain stimulation technique called transcranial magnetic stimulation (TMS) [14]. The motor-evoked potential (MEP) in a response to TMS, is measured in the electromyogram (EMG) of the target muscle and is used as a measure of corticospinal excitability or intracortical inhibition. In young adults, corticospinal excitability increases and intracortical inhibition decreases after motor practice [15, 16]. Neurochemical studies confirm this by showing a relationship between GABA decrease in the trained sensorimotor cortex and the magnitude of motor learning [17, 18].

In addition to excitability changes in specific brain areas such as the primary motor cortex, motor learning requires the involvement of a wide network of brain regions including cortico-cerebellar and cortico-striatal networks [19]. However, TMS only stimulates a focal brain area. Within 10 years after the implementation of TMS, it became possible to measure changes in brain activation by measuring the blood-oxygen-level dependent (BOLD) signal with functional magnetic resonance imaging (fMRI) [20-22]. This neuroimaging technique may provide more insight into the broad cortical and subcortical changes occurring after motor practice than TMS does. Because neurostimulation and neuroimaging techniques complement each other, in this thesis, we will use both TMS and fMRI to examine the neural mechanisms of motor learning.

1.3 Age-related changes in neural plasticity underlying motor learning

Increasing age is accompanied by impairments in the neuromuscular system such as sarcopenia [23], changes to peripheral nerve fibers [24], and a decrease in the number and increase in the size

of motor units [25, 26]. In addition, deteriorations in brain structure occur, including decreases in gray and white matter volume [27, 28], increases in cerebrospinal fluid volume [27], and decreases in regional white matter integrity [29]. Despite these age-related neural changes, older adults are still capable of learning new motor skills (see section 1.1). While the hypothesis that older adults use adaptive and perhaps compensatory neural strategies to sustain the ability to learn new motor skills is tenable, this has not been established. After acquiring a visuomotor tracking skill, corticospinal excitability increased and intracortical inhibition decreased independent of age [10]. However, others reported increases in corticospinal excitability in young but not in older adults after ballistic motor training [30]. Furthermore, whether or not age affects the nature and magnitude of synaptic plasticity accompanying motor memory consolidation remains unknown. Finally, neuroimaging studies showed greater and more widespread brain activation in old compared with young adults while executing motor tasks [31, 32] but it is unknown how age affects changes in brain activation patterns over the course of motor learning. Taken together, although there is some theoretical underpinning as to why adaptive strategies in the aging brain during motor learning are expected, it is not yet understood whether and how neural mechanisms of acquiring and consolidating motor skills into motor memory change with increasing age. A better understanding of the mechanisms of how age affects motor skill acquisition and consolidation would help design motor interventions counteracting age-related declines in motor function.

1.4 Outline and hypothesis thesis

The aim of this thesis is to examine age-related differences in the underlying neural mechanisms of motor learning. We used non-invasive neurostimulation (TMS) and neuroimaging (fMRI) techniques to measure markers of neural plasticity after both the acquisition and motor memory consolidation phase. Fig. 1 shows the visuomotor task that was used throughout the experimental chapters of this thesis.

In chapter 2, we examined how corticospinal and intracortical excitability at rest and during the execution of the task changed in healthy older adults after learning a visuomotor tracking task. Chapter 3 compares the data of healthy older adults obtained in chapter 2 with a group of young

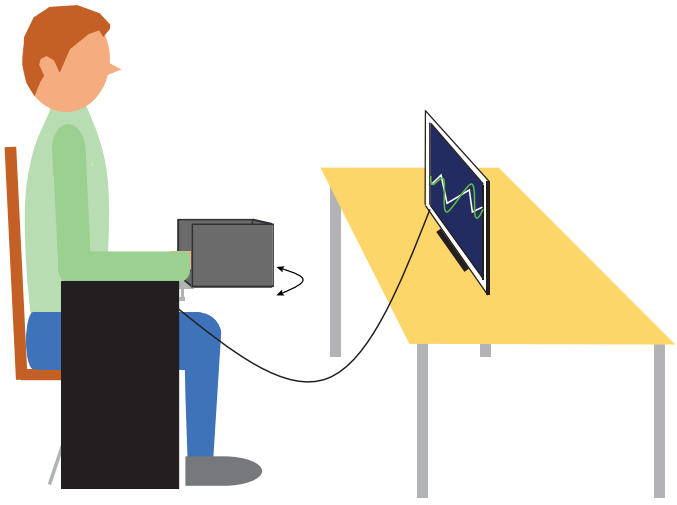

Fig. 1 Schematic representation of the set-up of the visuomotor task that was used in the experiments of chapters 2 and 3. Participants used wrist flexion and extension to track a zigzagged template (white) on the computer screen. Online feedback of participants' wrist position was provided (green). In Chapter 5, a similar task was used but participants laid supine in an MRI-scanner while performing the task.

adults to examine age-related differences in corticospinal and intracortical excitability after visuomotor learning. To give an overview of TMS studies regarding motor learning in aging, we conducted a systematic review and meta-analysis in chapter 4 and examined the relationship between motor skill acquisition and changes in TMS variables using individual data of the included studies. Because MEP measurements only provide indirect information about neural plasticity in the targeted primary motor cortex, we used fMRI in chapter 5 to examine age-related differences in brain activation changes in the whole brain after visuomotor learning. Finally, chapter 6 will provide a discussion of the results obtained in chapters 2-5 and will integrate these results with each other. We hypothesized that older adults would use alternative strategies of neural plasticity compared with young adults to learn new motor skills. These alternative strategies might be compensatory for age-related structural and functional changes in the brain.

References

1. Willingham DB. A neuropsychological theory of motor skill learning. Psychol Rev. 1998;105(3):558.

2. Dudai Y. The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol. 2004;55:51-86.

3. Krakauer JW, Shadmehr R. Consolidation of motor memory. Trends Neurosci. 2006;29(1):58-64.

4. Doherty TJ, Vandervoort AA, Brown WF. Effects of ageing on the motor unit: A brief review. Can J Appl Physiol. 1993;18(4):331-58.

5. Fjell AM, Walhovd KB. Structural brain changes in aging: Courses, causes and cognitive consequences. Rev Neurosci. 2010;21(3):187-221.

6. Aagaard P, Suetta C, Caserotti P, Magnusson SP, Kjaer M. Role of the nervous system in sarcopenia and muscle atrophy with aging: Strength training as a countermeasure. Scand J Med Sci Sports. 2010;20(1):49-64.

7. Chen YT, Kwon M, Fox EJ, Christou EA. Altered activation of the antagonist muscle during practice compromises motor learning in older adults. J Neurophysiol. 2014;112(4):1010-9.

8. Brown RM, Robertson EM, Press DZ. Sequence skill acquisition and off-line learning in normal aging. PLoS One. 2009;4(8):e6683.

9. Cirillo J, Rogasch NC, Semmler JG. Hemispheric differences in use-dependent corticomotor plasticity in young and old adults. Exp Brain Res. 2010;205(1):57-68.

10. Cirillo J, Todd G, Semmler JG. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. Eur J Neurosci. 2011;34(11):1847-56.

11. Rioult-Pedotti MS, Friedman D, Donoghue JP. Learning-induced LTP in neocortex. Science. 2000;290(5491):533-6.

12. Sanes JN, Donoghue JP. Plasticity and primary motor cortex. Annu Rev Neurosci. 2000;23:393-415.

13. Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. J Neurosci. 2004;24(7):1666-72.

14. Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet. 1985;1(8437):1106-7.

15. Perez MA, Lungholt BK, Nyborg K, Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. Exp Brain Res. 2004;159(2):197-205.

16. Jensen JL, Marstrand PC, Nielsen JB. Motor skill training and strength training are associated with different plastic changes in the central nervous system. J Appl Physiol (1985). 2005;99(4):1558-68.

17. Stagg CJ, Bachtiar V, Johansen-Berg H. The role of GABA in human motor learning. Curr Biol. 2011;21(6):480-4.

18. Floyer-Lea A, Wylezinska M, Kincses T, Matthews PM. Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. J Neurophysiol. 2006;95(3):1639-44.

19. Lohse KR, Wadden K, Boyd LA, Hodges NJ. Motor skill acquisition across short and long time scales: A meta-analysis of neuroimaging data. Neuropsychologia. 2014;59:130-41.

20. Bandettini PA, Wong EC, Hinks RS, Tikofsky RS, Hyde JS. Time course EPI of human brain function during task activation. Magn Reson Med. 1992;25(2):390-7.

21. Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, et al. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. Proc Natl Acad Sci U S A. 1992;89(12):5675-9.

22. Ogawa S, Tank DW, Menon R, Ellermann JM, Kim SG, Merkle H, et al. Intrinsic signal changes accompanying sensory stimulation: Functional brain mapping with magnetic resonance imaging. Proc Natl Acad Sci U S A. 1992;89(13):5951-5.

23. Doherty TJ. Invited review: Aging and sarcopenia. J Appl Physiol (1985). 2003;95(4):1717-27.

24. Verdu E, Ceballos D, Vilches JJ, Navarro X. Influence of aging on peripheral nerve function and regeneration. J Peripher Nerv Syst. 2000;5(4):191-208.

25. Brown WF, Strong MJ, Snow R. Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. Muscle Nerve. 1988;11(5):423-32.

26. Ling SM, Conwit RA, Ferrucci L, Metter EJ. Age-associated changes in motor unit physiology: Observations from the baltimore longitudinal study of aging. Arch Phys Med Rehabil. 2009;90(7):1237-40.

27. Coupe P, Catheline G, Lanuza E, Manjon JV, Alzheimer's Disease Neuroimaging Initiative. Towards a unified analysis of brain maturation and aging across the entire lifespan: A MRI analysis. Hum Brain Mapp. 2017;38(11):5501-18.

28. Ge Y, Grossman RI, Babb JS, Rabin ML, Mannon LJ, Kolson DL. Age-related total gray matter and white matter changes in normal adult brain. part I: Volumetric MR imaging analysis. AJNR Am J Neuroradiol. 2002;23(8):1327-33.

29. Salat DH, Tuch DS, Greve DN, van der Kouwe AJ, Hevelone ND, Zaleta AK, et al. Age-related alterations in white matter microstructure measured by diffusion tensor imaging. Neurobiol Aging. 2005;26(8):1215-27.

30. Rogasch NC, Dartnall TJ, Cirillo J, Nordstrom MA, Semmler JG. Corticomotor plasticity and learning of a ballistic thumb training task are diminished in older adults. J Appl Physiol (1985). 2009;107(6):1874-83.

31. Cabeza R, Anderson ND, Locantore JK, McIntosh AR. Aging gracefully: Compensatory brain activity in high-performing older adults. Neuroimage. 2002;17(3):1394-402.

32. Ward NS. Compensatory mechanisms in the aging motor system. Ageing Res Rev. 2006;5(3):239-54.

I Chapter 2 I

Neuronal mechanisms of motor learning and motor memory consolidation in healthy old adults

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Age (2015), 37:53

Abstract
It is controversial whether or not old adults are capable of learning new motor skills and consolidate the performance gains into motor memory in the offline period. The underlying neuronal mechanisms are equally unclear. We determined the magnitude of motor learning and motor memory consolidation in healthy old adults and examined if specific metrics of neuronal excitability measured by magnetic brain stimulation mediate the practice and retention effects. Eleven healthy old adults practiced a wrist extension-flexion visuomotor skill for 20 minutes (MP, 71.3 years), while a second group only watched the templates without movements (attentional control, AC, n=11, 70.5 years). There was 40% motor learning in MP but none in AC (interaction, $p<0.001$) with the skill retained 24 hours later in MP and a 16% improvement in AC. Corticospinal excitability at rest and during task did not change, but when measured during contraction at 20% of maximal force, it strongly increased in MP and decreased in AC (interaction, $p=0.002$). Intracortical inhibition at rest and during the task decreased and facilitation at rest increased in MP, but these metrics changed in the opposite direction in AC. These neuronal changes were especially profound at retention. Healthy old adults can learn a new motor skill and consolidate the learned skill into motor memory, processes that are most likely mediated by disinhibitory mechanisms. These results are relevant for the increasing number of old adults who need to learn and relearn movements during motor rehabilitation.

2.1 Introduction

Even healthy aging is associated with an up to 50% reduction in the number and diameter of motoneuron axons, a decrease in number of large-diameter axons, slowing of peripheral nerve conduction, impaired sensory fiber function, prolongation of reflex latencies, and a loss and subsequent remodeling of motor units [1]. Modifications in the peripheral nervous system are accompanied by substantial and functionally relevant reductions in gray matter volume in the primary motor, somatosensory cortices, and the cerebellum [2-5]. In addition to cortical atrophy, there are quantitative and qualitative changes in white matter structure and integrity (reviewed in [6, 7]). Such and other age-related changes in the neuromuscular system and a general reduction in motor activity make voluntary movements weak, slow, unsteady, and inaccurate [1, 8, 9]. With regard to the relatively well-characterized age-related changes in neuromuscular properties, a more contentious issue is whether or not healthy old adults can learn and retain new motor skills. Understanding the mechanisms of how and if age affects the ability to learn and re-learn motor skills is especially relevant because, with increasing age, more and more old adults receive movement rehabilitation that includes the learning and re-learning of movements impaired by specific comorbidities [10], as, for example, is the case after a stroke [11]. In addition, a better understanding of how healthy old adults learn and re-learn a novel motor skill is important because many old adults must operate and manipulate new electronic devices and need to acquire motor skills in new jobs [12, 13].

Despite the many unfavorable age-related changes in neuromuscular function and brain structures involved in motor learning, results from a group of studies provide evidence that age may not necessarily impair the ability to acquire novel motor skills [12, 14-17]. For example, old and young adults, practicing a visuomotor tracking task for 18 minutes, showed similar, about 23%, performance gains [18]. However, another group of studies reported that the ability to learn new motor skills in a single training session decreases with age [12, 14, 17]. To illustrate, the learning rate of a bimanual coordination pattern with 90° phase offset between the limbs is smaller in seniors compared with adolescents [17]. Finally, there is some evidence suggesting that performance gains in reaction time are actually superior in old compared with young adults [15].

In addition to the immediate performance gains, another important element of motor learning is the ability to retain and recall the previously acquired motor skills. Motor memory consolidation is the stabilization of memory traces following the initial online motor learning or acquisition period and can result in increased resistance to interference or even an improvement in performance after an offline period [19]. There is some evidence for an age-related decline in motor memory consolidation because old adults were able to stabilize the learned reaction time skills at the retention test 24 hours after the first training session (retention gain = -4.5 ms, $p > 0.05$), whereas young subjects showed not only stabilization but further improvements in the retained skills in the offline period (retention gain = 36.8 ms, $p < 0.01$) [15]. In other studies, reaction time improved after motor practice during the 12-hour offline period with greater gains in young compared with old adults [20, 21]. Young adults also showed improvements at 24-hour and 1-week retention test, whereas old adults did not [20, 21]. Furthermore, a recent study showed that memory consolidation of a ballistic wrist flexion skill is impaired with aging [16], and finally, sequence-specific knowledge

decreased between sessions in old but it stayed stable in young adults, suggesting weaker consolidation of sequence-specific knowledge in the elderly [21]. However, we must note the wide variation in methods that these studies used to examine motor learning and motor memory consolidation in aging.

There is a paucity of data concerning the underlying neuronal mechanisms involved in motor learning and motor memory consolidation in old adults. A transcranial magnetic stimulation (TMS) study compared corticomotor excitability and short-interval intracortical inhibition (SICI) between young and old adults after 300 rapid thumb abduction movements [22]. Old (124%) compared with young (177%) adults achieved lower gains in motor performance. Corticomotor excitability increased after motor practice in young but not in old subjects, and motor practice did not modify SICI in either age group. Practice of a complex visuomotor task in the form of index finger ab- and adduction improved task accuracy similarly in both age groups (7-24% range) with an increase in corticospinal excitability and reduction in SICI independent of age [18]. None of these studies examined motor learning, motor memory consolidation, as well as indices of neuronal mechanisms in combination in healthy older adults.

Changes in corticospinal excitability (CSE) measured at rest presumably reflect changes in long-term potentiation-like mechanisms involved in motor learning [23-25]. However, no studies have examined if changes in CSE after motor learning would also occur during task performance in old adults. Measurements at rest and during task performance seem intuitively and mechanistically warranted because these could reflect the activation of different portions of the motoneuron pool and also changes in the input-output gain of individual motoneurons or at the level of the motoneuron pool [26, 27]. In addition, SICI is a GABA-A-mediated inhibition that occurs in primary motor cortex (M1) circuits [28, 29], and its reduction is associated with the induction of long-term potentiation [30]. Measurement of SICI not only at rest, as it has been done in all previous motor learning studies using TMS, but also during the task itself would add to the mechanistic understanding of motor learning by increasing the specificity of measurements. Based on the mixed results reported previously concerning the changes in CSE and SICI at rest in young and old adults after motor learning [18, 22, 31], we favor the hypothesis that measurements of neuronal excitability when the muscle is active (i.e., during the task or a muscle contraction) are more sensitive and specific to motor learning than the same tests performed at rest after motor practice. This is because, after motor skill learning, there is an increase in brain activation in secondary motor areas, for example, pre-motor and supplementary motor areas (for a review, see [32]), making it likely that neuronal excitability measurements during contraction but not at rest would represent activity of secondary motor areas upstream M1.

The aim of this study was to determine the magnitude of motor learning and motor memory consolidation in healthy old adults and examine, for the first time, if specific metrics of motor cortical and corticospinal function measured by TMS mediate the practice and retention effects. Because motor learning is known to rely on attentional resources [32-34], our experimental approach controlled for the attentional load associated with motor practice, an element absent in previous studies.

2.2 Methods

2.2.1 Subjects

Twenty-two healthy older adults volunteered to participate in this study (14 men and 8 women; age, 70.9 ± 2.9 years; height, 1.74 ± 0.09 m; weight, 78.9 ± 15.3 kg; body mass index, 26.1 ± 5.3 kg/m^2). We evaluated subjects' health status using the Groningen Activity Restriction Scale (GARS), a reliable and valid test of disability in Activities of Daily Living (ADL) or Instrumental ADL (IADL) [35]. We assessed subjects' cognitive health with the Mini Mental State Examination (MMSE) [36]. Handedness was evaluated with the Edinburgh handedness inventory [37]. Subjects were excluded from the study if they suffered from neurological conditions, took medications influencing nerve conduction velocity, and had contraindications for the use of TMS, a pacemaker, metal in the brain or skull, and had uncorrected vision [38]. Subjects were also excluded if they had pain or movement constrictions in their right arm or hand. Subjects were asked not to consume coffee or tea an hour before the start of the experiment on each of the two testing days. Subjects signed an informed consent document, approved by the Medical Ethical Committee of the University Medical Center Groningen.

2.2.2 Procedure

Subjects were randomly assigned to one of two groups: motor practice group (MP) or attentional control group (AC). Testing procedure consisted of a pre-, post- and retention test (Fig. 1). Pre- and posttests were performed on Day 1 and the retention test was performed 24 hours later on Day 2. To control for variation in responses to TMS due to a diurnal effect, the retention tests were administered within ±30 minutes of the time when the pretest was administered 24 hours earlier, during the day between 9 AM and 3 PM. The design included a 24-hour retention interval, categorized normally as a delayed test [39]. The pretest consisted of TMS measurements at rest and during the motor task, peripheral nerve stimulation that determined the maximal compound action potential (M_{max}), hand function test, and the baseline assessment of visuomotor skill. TMS parameters included corticospinal excitability at rest (CSE) and during the visuomotor task (CSE_{task}), short-interval intracortical inhibition at rest (SICI) and during the visuomotor task ($SICI_{task}$), intracortical facilitation at rest (ICF) and during the task (ICF_{task}), cortical silent period (CSP), and contralateral facilitation (CLF) at 20% of maximal voluntary contraction (MVC). After the pretest, one of the two interventions was performed for a period of 20 minutes: Subjects either performed MP or AC. Subjects in MP performed the visuomotor task during the intervention period. The duration of the intervention was based on previous data suggesting that such a practice period is sufficient to reliably produce fast motor learning [18, 22]. Because motor learning is known to involve strong attentional elements [32-34], our design also included a group in which we assessed the magnitude of learning produced by attention to the task. Subjects in AC focused, during the intervention period, their attention on the visuomotor templates that appeared on the monitor but did not perform any movements. Instructions were as follows: "Follow the template only with your eyes but not with your hand." The posttest was a repeat of the pretest in both groups. On Day 2, sleep quality and quantity of the last month and last night were determined using the Pittsburgh Sleep Quality Index [40]. In addition, we repeated the pretest measurements to quantify the retention of motor memory traces and to determine the long-lasting changes in

measures of neuronal excitability.

In a control experiment conducted in additional five healthy, right-handed old adults (age, 69.8 ± 3.83 years), we examined the possibility that only familiarization of subjects with the motor task could produce learning and affects also retention. We also wished to quantify the variability in the TMS data by repeating these measurements three times. These subjects performed the same protocol as did the subjects in the main experiment, but instead of motor practice and attentional control, they sat for 20 minutes and read newspapers, using their left hand to turn pages.

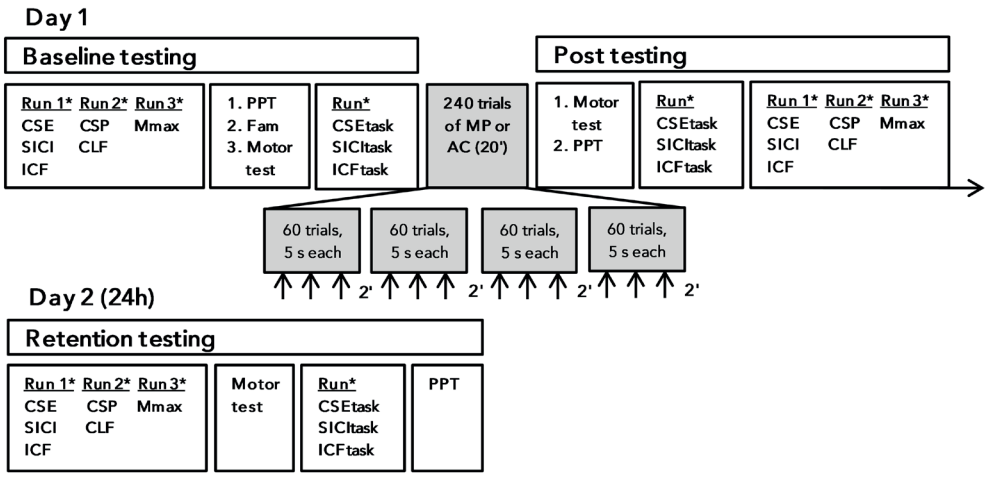

Fig. 1 The experimental design consisted of the pre- and posttests on Day 1 and a retention test on Day 2. Upward directed arrows indicate the time when subjects performed a counting task to control for attentional drift. The order of the runs within a block and the order of the pulses within a block were randomized (*). Abbreviations: AC, attentional control; CLF, contralateral facilitation; CSE, corticospinal excitability; CSE_{task}, corticospinal excitability during task; CSP, cortical silent period; Fam, familiarization; ICF, intracortical facilitation; ICF_{task}, intracortical inhibition during task; M_{max}, maximal compound action potential; MP, motor practice; PPT, Purdue Pegboard test; SICI, short-interval intracortical inhibition; $SICI_{task}$, short-interval intracortical inhibition during task.

2.2.3 Behavioral testing and motor practice

Subjects sat comfortably in a chair without armrests approximately 90 cm in front of a laptop computer's monitor (diagonal distance 39.6 cm). Their right forearm was fixed in a padded manipulandum in a neutral wrist position, the thumb pointing upwards. The center of the wrist joint was aligned with the axis of the manipulandum that confined wrist motion to flexion and extension. The left arm was resting on a table covered with soft material in a pronated position. The knees were flexed 90° and the feet were flat on the floor.

As reported previously, we used a visuomotor task for behavioral testing and also for the motor practice intervention, consisting of template tracking [18, 41, 42]. Subjects were asked to match the template as accurately as possible by flexing and extending the right wrist. The template

appeared on the monitor, proceeded from left to right, and changed direction that prompted wrist extension (template up) and flexion (template down). The background on the monitor was dark blue and contained a hairline-thick light blue-colored grid. The template appeared in white and the subject's performance line appeared in green color in high resolution.

Trials used for testing subjects' visuomotor skill consisted of six templates of different patterns. Templates were scaled to each subject's wrist range of motion. Trials used for the interventions also consisted of six different template patterns. Templates used for the interventions and the templates used to assess learning were different but were of similar difficulty as quantified by the number of turns. There were one or two turns within each template, i.e., changes in direction (mean, 1.33 ± 0.49). The order and duration of the templates were randomized but was the same for each subject at the three tests. The duration of the templates varied between 4, 5, or 6 seconds (mean, 4.99 ± 0.82s).

Prior to testing, subjects performed three familiarization trials. Next, they completed 12 pretest trials to establish baseline. After this pretesting, MP completed 4 blocks of 60, a total of 240 trials. After every 15 trials, subjects in both groups were asked to count backwards by seven to minimize attentional drift. Between training blocks, subjects in both groups rested for 2 minutes. After the interventions, subjects repeated the same 12 trials used in the pretest to assess the magnitude of motor learning. On Day 2, a retention test containing 12 trials was administered.

2.2.4 Hand function

In order to determine if the acquisition and/or motor memory consolidation of the visuomotor skill transferred to a nonpracticed motor task, i.e. a task variant, the Purdue Pegboard test was administered at baseline and after motor practice and attentional control on Day 1 and also on Day 2 during the retention test [43]. The Purdue Pegboard test reliably measures gross motor movements of the arms, hand, and fingers and fine motor dexterity [44, 45].

2.2.5 EMG recording

Subject's skin was prepared for electromyography (EMG) by shaving, scrubbing with fine sandpaper, and cleaning the skin with alcohol to minimize noise in the EMG signal. EMG was recorded in the left and right flexor carpi radialis (FCR) and left and right extensor carpi radialis (ECR) and using 37x27x15mm, <15g, wireless, preamplified (909x) parallel-bar sensors, affixed to the skin with a four-slot adhesive skin interface (Trigno, Delsys Inc, Natick, MA, USA). The electrodes recorded with a bandwidth of 20-450 Hz, channel noise <0.75 μV, and common mode rejection ratio >80 dB. EMG activity was sampled at 4 kHz. Signals were acquired online and stored by software installed on a personal computer for offline analysis (Power 1401 and Signal, Cambridge Electronics Design, Cambridge, UK).

2.2.6 Transcranial magnetic stimulation

Single- and paired-pulse TMS measurements were performed with two Magstim 200 magnetic stimulators (Magstim Company Ltd, Dyfed, UK). A figure of eight coil (loop diameter, 90 mm) was connected to BiStim^2 stimulators and held over the optimal stimulation spot of the left motor cortex to elicit motor-evoked potentials (MEPs) in the right ECR with the handle pointing backwards at

~45° away from the sagittal plane. To ensure consistent coil position during the experiments, the optimal point, the hot spot, for stimulating the right ECR was marked on a cloth cap that the subjects wore. Resting motor threshold (RMT) was defined as the minimum intensity (% stimulator output) where five out of the 10 trials evoked an MEP in the right ECR with amplitude ≥ 50μV [46, 47]. Additionally to RMT, in nine subjects, active motor threshold (AMT) was measured, defined as the minimum intensity (% stimulator output) where five out of the 10 trials evoked an MEP in the right ECR with amplitude ≥200 μV and above-background EMG signal during isometric contraction of the right ECR at 10% MVC [48].

CSE, SICI and ICF were determined at rest. Test pulse was set at 120% RMT, and conditioning pulse was set at 80% RMT [29]. The interval between the paired-pulses for determining SICI and ICF were, respectively, 2 and 10 ms [29]. Subjects received a total of 30 pulses, randomized 10 single pulses, 10 paired pulses with 2-ms interval, and 10 paired pulses with 10-ms interval.

CSE [49-52], SICI and ICF were also measured during the visuomotor task (CSE_{task}, $SICI_{task}$ and ICF_{task}) in nine subjects. Subjects completed 30 trials of the visuomotor task. These trials started with a flexion followed by an extension movement but still had an element of difficulty because there were five different templates appearing in a random order. During the extension phase of the trial as the wrist passed at 8° extension, subjects received randomized 10 single pulses, 10 paired pulses with 2-ms interval, and 10 paired pulses with 10-ms interval. Conditioning pulse was set at 70% AMT and test pulse at 120% AMT [53].

CSP and CLF were measured to determine motor cortical inhibition and facilitation during weak muscle contraction specific to the task. Subjects received 15 TMS pulses at 120% RMT. The first five pulses subjects had both arms in rest, but during the next 10 pulses, subjects performed an isometric contraction at ±8° into wrist extension at 20% MVC. CSP is the interruption of ongoing EMG activity after a TMS pulse is given [54].

2.2.7 Peripheral nerve stimulation

M_{max} was defined as the maximal peak-to-peak amplitude of the M-wave as a response to electrical stimulation of the right radial nerve above the elbow. An electrical stimulator delivered the 0.5-ms-long square-wave stimulus (DS7A, Digitimer Ltd, Welwyn Garden City, UK). The stimulation intensity was increased until the peak-to-peak amplitude of the M-wave did not increase any further and then stimulation intensity was raised by 20% to ascertain M_{max}.

2.2.8 Data analysis

Matlab R2011a was used to analyze the behavioral data, i.e., the performance on the visuomotor task, and the CSP data (The Mathworks Inc., Natick, MA, USA). Visuomotor skill was determined by calculating the mean error of the subject's wrist joint position from the white preprogrammed template. The first second of the behavioral data was discarded because it contained errors associated with reacting to the appearance of the template. CSP onset, offset, and duration were determined using an adjusted version of the Teager Kaiser Energy Operator (TKEO), a highly effective method used to determine the boundaries of an EMG burst [55]. Signal 5.04 was used to analyze the remaining TMS parameters. Peak-to-peak amplitudes of MEPs were calculated in

order to determine CSE, CSE_{task}, SICI, $SICI_{task}$, ICF, ICF_{task}, and CLF. CSE and CSE_{task} were expressed by the MEP amplitude as a percentage of M_{max}. SICI and ICF at rest and during the task were expressed by the conditioned MEP as a percentage of the test MEP. CLF was defined as the mean peak-to-peak MEP amplitude of the trials with 20% MVC expressed as a percentage of the mean peak-to-peak MEP amplitude of the trials in rest. The background EMG activity was calculated as the mean rectified EMG activity in the period 70 ms before the TMS test pulse.

2.2.9 Statistical analyses

Data are reported as mean ± SD. Two-way repeated measures analysis of variances (ANOVA) was performed to determine the effects of intervention (MP, AC; between-subjects factor), time (baseline, posttest, retention at 24 h; within-subjects factor), and interactions of intervention and time on visuomotor skill, Purdue Pegboard performance, M_{max}, RMT, AMT, CSE, CSE_{task}, SICI, $SICI_{task}$, ICF, ICF_{task}, CLF, and CSP. When there was a between-group difference at baseline, an analysis of covariance (ANCOVA) was performed, using baseline values as a covariate. Tukey's post-hoc analysis was performed to determine the means that were different from one another. In the control experiment, we performed one-way repeated measures ANOVAs to determine if there was a main effect of time in each dependent variable.

In order to determine if baseline values and changes in visuomotor skill were associated with Purdue Pegboard performance and TMS variables (CSE, CSE_{task}, SICI, $SICI_{task}$, ICF, ICF_{task}, CLF, and CSP), Pearson's correlations were computed. For all analyses, we set the level of significance at $p < 0.05$.

2.3 Results

Table 1 shows that the 11 subjects (7 M and 4 F) in MP and AC were similar in age, MMSE, laterality score, GARS, PSQI, and the quantity and quality of sleep the night before testing. The 11 subjects (7 M and 4 F) in AC vs. MP were somewhat heavier and taller.

2.3.1 Behavioral data

Fig. 2 shows the group × time interaction in the amount of error ($F_{2, 40} = 12.3$, $p = 0.000$). With the two groups producing similar amount of error at baseline (difference, 1.9°, n.s.), after intervention, the reduction in error from baseline to posttest was 40% or 7.3° in MP ($p < 0.05$) and 6% or 1.3° in AC. At retention, MP maintained the posttest error level (0.6° more error, n.s.), while, relative to baseline, the error in AC decreased by 16% or 2.9° ($p < 0.05$, relative to baseline). From baseline to retention, the reduction in error was greater in MP (37% or 6.7°) compared with AC (21% or 4.2°). The control group had an error of 14.8° (± 2.0°) at baseline and showed a borderline time effect ($p = 0.056$). Error decreased by 2.8° due to familiarization with the task and increased 0.1° 24 hours later at retention.

There was a group × time interaction in the performance of the Purdue Pegboard test ($F_{2, 40} = 8.3$, $p = 0.001$). Pegboard performance did not improve in MP (baseline, 13.3 ± 1.2 pins; after motor practice, 13.6 ± 1.4 pins; retention, 13.5 ± 1.4 pins). AC compared with MP placed 1.5 more pins on the board at the retention test (baseline, 13.6 ± 1.9 pins; after template viewing, 14.9 ± 1.8 pins; at retention, 15.0 ± 2.1 pins). Pegboard performance was stable in the control

experiment (baseline, 13.0 ± 2.6; posttest, 13.4 ± 3.1; retention, 13.6 ± 2.3 pins; p = 0.41).

Table 1. Characteristics of subjects in the motor practice group (MP, n = 11) and attentional control group (AC, n = 11).

Variable	MP, mean (±SD)	AC, mean (±SD)
Age (years)	71.3 (3.35)	70.5 (2.50)
Mass (kg)	73.3 (9.34)	84.5 (18.32)
Height (m)	1.71 (0.10)	1.77 (0.07)
BMI (kg/m^2)	24.9 (1.92)	27.4 (7.20)
MMSE	28.7 (1.74)	29.4 (1.00)
GARS	18.4 (1.21)	18.1 (0.30)
Laterality quotient	0.91 (0.09)	0.96 (0.08)
PSQI	5.2 (4.29)	5.0 (3.97)
Quantity of sleep (h)	6.7 (1.69)	7.2 (0.94)
Quality of sleep[a]	1	1

Abbreviations: BMI, body mass index; MMSE, Mini Mental State Examination (> 27 cognitively healthy); GARS, Groningen Activity Restriction Scale (18–72, the higher the score, the higher the activity restriction); PSQI, Pittsburgh Sleep Quality Index (lower score is higher quality of sleep in last month); Quantity of sleep in hours the night before retention testing; Quality of sleep on a scale from 0 (best) to 3 (worst) in the night before retention testing
[a] Instead of mean (± SD), the modus is shown for the results of this 4-point Likert-scale

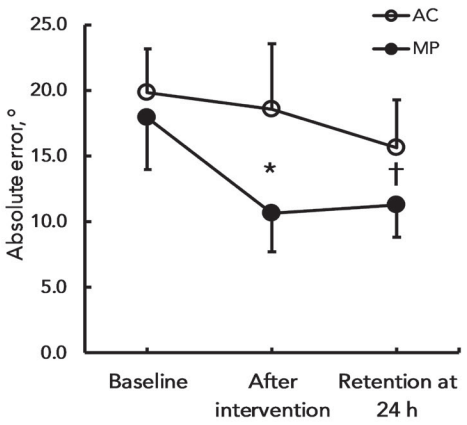

Fig. 2 Motor learning data. The magnitude of error in the two groups was similar at baseline. After active motor practice (filled symbols), the magnitude of error was significantly lower compared with baseline and compared with attentional control (open symbols, *). After 24 hours, the magnitude of error after attentional control was lower compared with baseline but greater than after motor practice (†). Vertical bars denote ±1 standard deviation.

2.3.2 Peripheral nerve stimulation

Supramaximal stimulation of the radial nerve consistently evoked an M_{max} with similar peak-to-peak amplitudes at baseline (MP, 2.4 ± 0.75 mV; AC, 2.1 ± 0.78 mV), after interventions (MP, 2.4 ± 0.89 mV; AC, 2.3 ± 0.74 mV), and at retention (MP, 2.4 ± 0.74 mV; AC, 2.3 ± 0.79 mV), resulting

in no group × time interaction ($p = 0.541$) or a time main effect ($p = 0.623$). There was also no main effect of time in the control group (baseline, 2.7 ± 1.9; posttest, 2.7 ± 2.1; retention, 2.3 ± 1.3 mV; $p = 0.465$).

2.3.3 Brain stimulation data

Table 2 shows the resting and active motor threshold and the corticospinal excitability data at rest and during the visuomotor task, normalized and not normalized for M_{max}, and corticospinal excitability data during an isometric wrist extension at 20% MVC normalized for MEP amplitudes in rest. The group × time interactions and the time main effects were not significant for RMT, AMT and corticospinal excitability at rest and during the visuomotor task (all effects $p > 0.05$). However, there was a group × time interaction for contralateral facilitation measured as the facilitation of a standard motor evoked potential delivered at 120% of RMT during a wrist extension at 20% isometric MVC ($F_{2, 40} = 7.6$, $p = 0.002$, see Table 2). Facilitation was similar at baseline (MP, 340.7% ± 148.7; AC, 386.3% ± 159.9, $p > 0.05$). These data mean that the wrist extension at 20% MVC facilitated the MEP measured at rest by 3.4- and 3.8-fold in MP and AC, respectively. Motor practice increased this facilitation to 400.2% (± 187.0), while the facilitation decreased to 329.2 (± 109.5) in AC (both $p < 0.05$). At retention, the facilitation further increased in MP (627.0 ± 364.8) and further decreased in AC (292.2% ± 106.6) (both $p < 0.05$). The difference in contralateral facilitation was 71% after the intervention and 335% at retention, with the facilitation being higher in MP vs. AC ($p < 0.05$). Thus, corticospinal excitability during a wrist extension at 20% isometric MVC increased in MP but decreased in AC.

Fig. 3 shows representative examples of SICI measured at rest in one subject in MP and one AC subject, and Fig. 4 shows the group data of SICI and ICF. Fig. 4a shows the group × time interaction for SICI recorded at rest ($F_{1.488, 28.272} = 4.6$, $p = 0.027$). The value of SICI was 52.1% (± 28.0) and 54.1% (± 14.0) in MP and AC, respectively, at baseline. After the interventions, the corresponding values in MP and AC were 57.1% (± 13.0) and 47.2% (± 22.0) ($p < 0.05$). After the interventions, nine of 11 subjects had less intracortical inhibition in MP, and nine of 11 subjects had more intracortical inhibition in AC. At retention, SICI was 73.5% (± 27.7) in MP and 43.7% (± 26.6) in AC (both between-group differences and relative to baseline $p < 0.05$). At retention, 10 of 11 subjects had less intracortical inhibition in MP, and 8 of 11 subjects had more intracortical inhibition in AC. Thus, intracortical inhibition decreased after MP, but it increased after AC.

Fig. 4b shows the group × time interaction ($F_{2, 40} = 4.0$, $p = 0.026$) for $SICI_{task}$. As expected, the baseline values of $SICI_{task}$ were higher (88.4% ± 11.4) than SICI (53.1% ± 21.0), suggesting lower intracortical inhibition during contraction. The mean background EMG activity in the right ECR was 7.2% (± 3.2, MP) and 5.7% (± 2.7, AC, $t_{20} = 0.83$, $p = 0.237$) of the EMG activity measured in the ECR during a maximal effort isometric wrist extension. With similar $SICI_{task}$ values at baseline (MP, 86.1 ± 9.6; AC, 90.6 ± 13.2), the value of $SICI_{task}$ remained unchanged after MP (87.5% ± 16.2) but decreased after AC (83.7% ± 8.2). At the retention test, the value of $SICI_{task}$ increased in the MP group to 100.0% (± 20.8), while it remained the same in AC (83.5% ± 13.3), resulting in a between-group difference of 16.5% in the value of $SICI_{task}$ at retention ($p < 0.05$). Thus, intracortical inhibition decreased in MP and increased in AC both at rest and during the task, with the difference being especially prominent at retention.

Table 2. Effects of motor practice and attentional control on corticospinal excitability.

	Baseline, mean (±SD)	After intervention, mean (±SD)	At retention, mean (±SD)
RMT (% SO)			
Motor practice	54.2 (10.9)	55.6 (12.5)	56.0 (14.2)
Attentional control	51.0 (10.3)	51.4 (11.4)	52.8 (11.7)
AMT (% SO)			
Motor practice	50.4 (12.2)	45.6 (12.9)	51.2 (20.6)
Attentional control	47.8 (6.8)	47.3 (6.8)	46.8 (3.5)
CSE (mV)			
Motor practice	0.35 (0.29)	0.39 (0.30)	0.26 (0.25)
Attentional control	0.30 (0.24)	0.27 (0.14)	0.26 (0.10)
CSE (% M_{max})			
Motor practice	15.5 (11.4)	16.7 (15.4)	11.6 (10.8)
Attentional control	14.6 (9.7)	12.3 (5.9)	13.3 (3.7)
CSE_{task} (mV)			
Motor practice	1.01 (0.41)	1.01 (0.34)	0.77 (0.41)
Attentional control	1.05 (0.47)	0.96 (0.32)	0.92 (0.43)
CSE_{task} (% M_{max})			
Motor practice	47.6 (30.2)	47.4 (26.4)	34.7 (19.6)
Attentional control	55.0 (24.7)	45.8 (26.0)	43.5 (22.5)
CSE during 20%MVC (% MEP_{rest})			
Motor practice	340.7 (148.7)	400.2 (187.0)[a,b]	627.0 (364.8)[a,b]
Attentional control	386.3 (159.9)	329.2 (109.5)[b]	292.2 (106.6)[b]

Abbreviations: RMT, resting motor threshold; AMT, active motor threshold; CSE, corticospinal excitability; %SO, percent of stimulator output

[a] Group × time interaction ($F_{2, 40}$ = 7.6, p = 0.002)

[b] Facilitation increased in MP and decreased in AC relative to baseline with facilitation higher in MP than in AC after interventions and also at retention (all $p < 0.05$).

We also measured the contralateral silent period during wrist extension at 20% MVC. There was no group × time interaction ($F_{2, 40}$ = 1.7, p = 0.200) or a time main effect ($F_{2, 40}$ = 1.9, p = 0.163). Pooled across the three time points, the average duration of the net silent period was 75.5 ms (± 22.7) in MP and 71.0 ms (± 16.5) in AC (t-test: p = 0.368, data not shown).

Fig. 4c shows the borderline group × time interaction for intracortical facilitation measured at rest ($F_{2, 40}$ = 3.1, p = 0.054). The two groups were similar at baseline (MP, 140.6% ± 20.9; AC, 133.2 ± 35.7), but ICF tended to increase in MP (153.3% ± 33.0) and decrease in AC (118.6% ± 33.4), a trend that continued at the retention test in MP but not in AC (MP, 166.9% ± 35.4; AC, 124.5% ± 36.9). ICF_{task} did not change (group × time interaction, p = 0.181, data not shown).

The control experiment revealed no time main effects for any of the TMS variables with the p-values for the one-way repeated measures ANOVAs ranging from p = 0.143 to p = 0.874 (detailed data not shown).

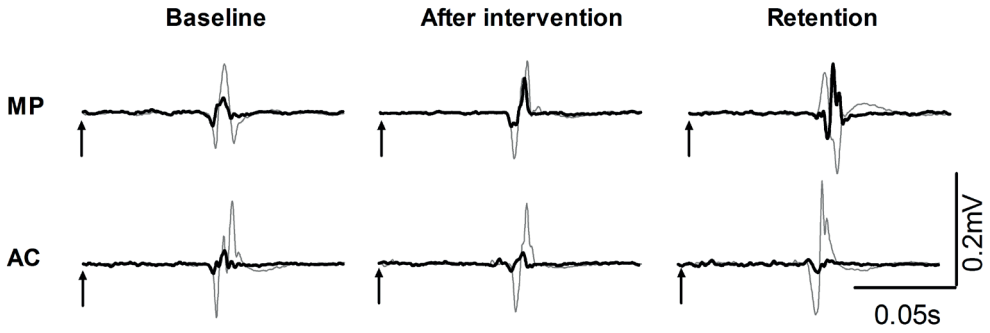

Fig. 3 Representative responses to transcranial magnetic stimulation in the right extensor carpi radialis muscle for one 68-year-old female subject in the motor practice and in one 70-year-old female subject in the attentional control group. Recordings were made at rest at baseline, after intervention, and at retention. Waveforms represent average of five motor evoked potentials in response to single test pulses (thin gray line) and conditioned pulses (thick black line) at an inter-stimulus interval of 2 ms. Arrows indicate when the test pulse is given.

2.3.4 Correlation analyses

Baseline levels and changes in visuomotor task and in the Purdue Pegboard test did not correlate in MP, AC, and in the two groups combined (21 r-values, $p > 0.05$). Changes in SICI measured at rest positively correlated with learning in MP ($r = 0.64$, $p < 0.05$) but not with the changes measured at retention ($p > 0.05$) (Fig. 5a). In contrast, changes in $SICI_{task}$ in MP negatively correlated with learning ($r = -0.59$, $p < 0.05$) but not with the changes measured at retention (Fig. 5b). These results indicate that an increased motor performance in MP is associated with more intracortical inhibition at rest and less intracortical inhibition during the task. None of these correlations were significant in AC.

2.4 Discussion

We observed 40% motor learning after only 20 minutes of practice of a visuomotor task, a skill that naive healthy old adults were able to consolidate into motor memory 24 hours later. In contrast, watching the same templates without actual movements produced no learning (6%, n.s). Corticospinal excitability at rest and during the visuomotor task remained unchanged in MP and AC but became strongly modified when measured during 20% MVC. Intracortical inhibition at rest and during the task decreased, and facilitation at rest increased after MP. TMS metrics changed in the opposite direction in AC. Only in a few of these metrics did the changes correlate with changes in behavior. The findings partially support the global hypothesis that neuronal measurements in an active state vs. at rest are more selective and sensitive to motor learning and retention. We discuss the data in the context of how motor cortical disinhibition may play a key role in motor learning and motor skill consolidation in the healthily aging motor cortex.

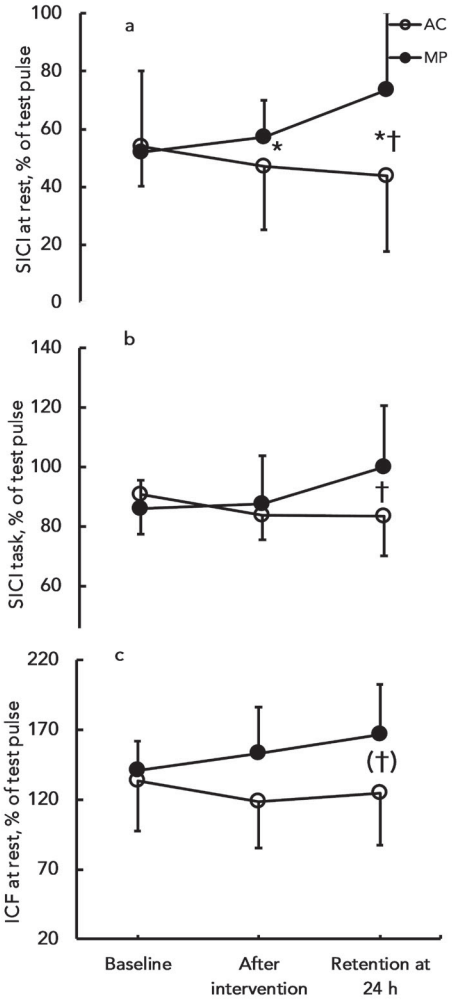

Fig. 4 Effects of motor practice and attentional control on short-interval intracortical inhibition at rest (**a**), measured during the task (**b**), and intracortical facilitation measured at rest (**c**). **a** Group × time interaction ($F_{1.488, 28.272} = 4.6$, $p = 0.027$). * $P < 0.05$ between groups and † $p < 0.05$ relative to baseline. **b** Group × time interaction ($F_{2, 40} = 4.0$, $^{\dagger}p = 0.026$). **c** Borderline group × time interaction ($F_{2, 40} = 3.1$, $p = 0.054$). SICI values < 100% indicate inhibition, and ICF values > 100% indicate facilitation. Filled and open symbols represent motor practice and attentional control, respectively. Vertical bars denote ±1 standard deviation.

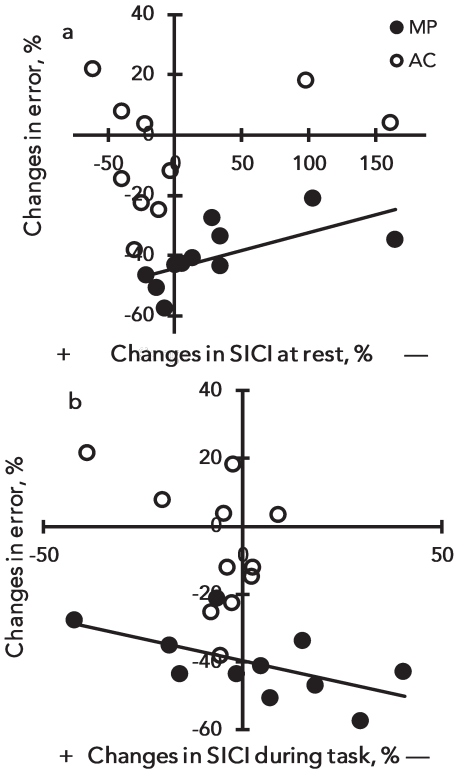

Fig. 5 Correlation between percent changes in intracortical inhibition (SICI) and visuomotor skill in the motor practice group (filled symbols) and attentional control group (open symbols). Correlations are shown between **a** changes in SICI values at rest and changes in error (MP: $R^2 = 0.41$, $y = 0.12x - 44.2$; AC: $R^2 = 0.08$, $y = 0.08x - 6.7$), and **b** changes in SICI values during task and changes in error (MP: $R^2 = 0.34$, $y = -0{,}26x - 39.7$; AC: $R^2 = 0.18$, $y = -0.61x - 10.3$). The positive and negative sign denotes, respectively, more or less inhibition.

2.4.1 Skill acquisition

Old adults are normally able to learn a novel motor task. However, when compared with young adults, the results can be inconsistent as learning can be similar [16, 18], become compromised [12, 14, 17, 22], or can even exceed young adults' scores [15]. Using models of error-based, reinforcement, and use-dependent learning [56], previous studies in healthy old adults reported 17-124% learning [6, 14, 16, 18, 22, 57], reflecting the fast phase of motor learning [32, 58]. The 40% learning after just 20 minutes of motor practice in the present study is well beyond the 24% reported in similar subjects, learning task, and exposure duration (18 minutes) but assessed in the index finger [18] (Fig. 2). Perhaps our task was more complex and represented a higher motor challenge compared with the finger [18] and therefore had more room for improvement. We note that, even though the 40% learning exceeds learning rates reported in this study [18], it is possible that there was actually even greater learning in MP because 20 minutes of motor practice can cause a saturation effect and mask a portion of learning [59, 60]. Previous studies reported ~24% learning after ~22 minutes of template tracking task in the finger (~24%, 18 minutes) [18], ankle (~35%, 32 minutes) [41], and elbow joint (~12%, 16 minutes) [42] in young adults, suggesting that our old adults acquired the skill at the wrist as well if not more proficiently than young adults. This finding qualitative agrees with previous studies [15, 18] but warrants some caution because there is a growing concern that the young-old comparisons are misleading or even invalid when the baseline values are different in the two age groups, a factor that also guided our choice of experimental design [61]. Another complicating factor that warrants caution is that the difficulty of the task templates in the current study differed from previous research. The large amount of learning did not transfer to a task variant because Pegboard scores remained unchanged, and the changes in the learned and the transfer task did not correlate ($r = 0.14$, n.s.). We suspect that transfer did not occur because the learning exposure was too short and early learning processes, albeit engaged in transfer, act ineffectively over such a time scale [6], and because placing the pins requires movements around all three axes of the wrist joint and of the fingers while the learning task was confined to wrist movements in the transverse plane and excluded the fingers. Overall, our data provide evidence that healthy old adults retain the ability to acquire a novel visuomotor skill with high proficiency using wrist flexion-extension but with a low generalization to a task variant.

2.4.2 Neuronal mechanisms of skill acquisition

Although we observed 40% motor learning after motor practice and no learning as a result of visually following the same templates on the computer screen, a global measure of neuronal excitability, resting (53% stimulator output) and active (49% stimulator output) motor threshold, and a marker of use-dependent plasticity, i.e., MEP size at rest (0.33 mV) and during the execution of the task (1.03 mV) remained all unchanged (Table 2). Most often, a lack of change or a reduction in MEP size after motor practice is interpreted as evidence for aberrations in long-term-potentiation-like mechanisms involved in experimentally induced and use-dependent motor memory formation in aging humans [62-66]. While age can certainly compromise M1's ability to reorganize in response to motor practice [67, 68], we favor the interpretation of our MEP data to simply signify a dissociation between learning and one particular measure of plasticity. While short-term error-based visuomotor learning tends to increase MEP size in young adults [41, 42,

69], a dissociation was also reported in young subjects performing an interleaved form of motor practice [69] and also in old adults who improved ballistic thumb abduction performance by 124% but without changes in MEP size [22]. As in the present study, learning outcomes after index finger practice also did not correlate with changes in MEP size in young and old adults [18]. In young subjects, such associations were also not reported or found after one session of visuomotor practice in the ankle [41] and elbow joint [42], and under certain conditions of serial reaction time task learning in the index finger [70]. Even after 13 sessions of visuomotor elbow joint practice, associations were not higher than $R^2 = 0.236$ [42]. It is possible that TMS accessed a different population of cells within the corticospinal path than the ones that were active during learning, an interpretation supported by animal data describing task-specific and selective activation of corticospinal neurons [71, 72]. Compared with previous motor learning studies, we increased the specificity of the corticospinal measurements by assessing in old adults for the first time MEP size during the task itself but, as at rest, found no adaptations in this metric either, an observation that was not consistent with our hypothesis. However, when the contraction was stronger (20% MVC) than during the task (5-7% MVC), corticospinal excitability assessed by the contralateral facilitation test increased from 340% (±148.7) to 400% (±187.0) in MP and decreased in AC ($p < 0.05$, Table 2), data that are compatible with the hypothesis.

Because muscle contraction ≥20% MVC compared with rest and weak contractions nonlinearly increase the magnitude and number of descending volleys during TMS, the contralateral facilitation data reflect how motor practice modified the contributions of the different early-phase I waves to the MEP [26]. With contraction, adaptations most likely occurred through a summation of I1 and I2 waves. At rest and during weak contractions, a summation of I1-I4 wave is needed to produce MEPs [26, 27]. These data suggest that adaptation in specific portion of the corticospinal neurons occurred when corticospinal excitability is tested at 20% MVC. The increased MEP at 20% MVC in MP could also reflect a modulation of the input-output gain of individual motoneurons or at the level of the motoneurons pool [73]. Collectively, the single-pulse TMS data suggest that, except for adaptations at stronger background contractions, indices of corticospinal excitability at rest and during the task were, in contrast with the hypothesis, under the present experimental conditions perhaps not sensitive, selective, or specific enough to detect changes normally used to index use-dependent plasticity after motor learning.

Intracortical inhibition at rest and during the task decreased and facilitation at rest increased after motor practice, but these outcomes changed in the opposite direction after the attentional control intervention (Fig. 4). SICI is a GABA-A-mediated inhibition that occurs in M1 circuits particularly affecting I3 waves [28, 29], and, as demonstrated in slices prepared from the rodent primary motor cortex [74, 75], its reduction is associated with the induction of long-term potentiation, a process involved in motor learning [30, 76].

In humans, intracortical inhibition indexed with SICI has, however, revealed somewhat inconsistent changes after motor practice: It decreased [18, 41, 69, 77-82] or remained unchanged in young and old subjects [22, 31]. While corticospinal excitability data obtained through our single-pulse experiments increased only during 20% MVC in MP (Table 2), our double-pulse SICI data at

rest and during task agree with the trend for disinhibition acting as a mediating mechanism of improved performance after motor practice in old adults. The moderate negative association ($r = -0.59$, $p = 0.043$) between increase in motor performance and decrease in inhibition measured during the task assigns, as hypothesized, a functional role to disinhibition measured at least during the task (Fig. 5b). However, the direction of this association was positive at rest ($r = 0.64$, $p < 0.034$, Fig. 5a), suggesting a different role or involvement of these circuits at rest than during the task, a finding future studies will have to confirm. Based on the current data, we are unable to disentangle whether the reduction in SICI measured during the task in MP is the result of a reduction in cortical GABAergic inhibition or a superimposition of a concurrent facilitation recruited during task contraction [53]. Because our recording conditions (5-7% MVC during the task, 2-ms interstimulus interval, conditioning pulse of 70% AMT) were similar under which previously "pure" SICI was identified, we favor the interpretation that a superimposition of short-interval intracortical facilitation on SICI played a small or no role in the SICI reductions in MP [53] (Fig. 4a, b). We also note that neither intervention affected ICF during the task, and there was only a borderline group × time interaction at rest driven by the retention but not the post-intervention data (cf. [41], Fig. 4c), suggesting a putative role for reduced GABA-A inhibition instead of facilitatory mechanisms mediating motor learning under these conditions. A lack of changes in contralateral silent period, a measure of GABA-B function [83], further highlights the GABA-A system involvement.

2.4.3 Skill retention

A few studies in old adults examined the retention of a learned skill 24 hours after practice, using models of error-based, reinforcement, and use-dependent learning [12, 15-17, 20, 21] but none with the template-matching error-based model. The pattern of no additional improvement but stabilization of the learned skill in the present study qualitatively agrees with the -10 to 10% 24-hour change reported in these studies (but see [14]). While motor skill acquisition occurs online, stabilization, and further improvements in the skill, and a reduction in the fragility of the motor memory traces are the results of offline processes [84-88] that allow the consolidation of the skill into motor memory [23, 89, 90]. Sleep can affect motor memory consolidation induced by error-based explicit motor learning under some [88] but not all conditions [60]. The quantity and quality of sleep was similar in MP and AC, making it unlikely that differences in these measures of sleep would have caused the observed differences in motor learning, retention, and neuronal excitability between the two groups.

Several of the TMS metrics revealed amplified changes at retention compared with the data after the interventions, recorded 24 hours earlier. We are not aware of any previous studies in healthy young or old adults reporting TMS data at 24 hours after motor practice. During the offline period after the motor practice to retention, there was a continued reduction in SICI measured at rest and during the task and an increase ICF at rest (borderline), and strong additional increases in contralateral facilitation measured during 20% MVC. The absence of correlations between the changes in these TMS metrics and learning outcome at retention suggest that memory trace stabilization was perhaps the result of neuronal processes other than the ones we measured, using the TMS metrics included in the study design (correlations not shown). This speculation is reinforced by the data seen in AC: There were significant improvements during offline period

with a downward and opposite trend in the TMS metrics (Figs. 2 and 4). As in AC in the present study, finger-tapping practice in the sham control group in a previous tDCS study produced no learning, but performance increased at the 90-minute retention test [12]. However, the neuronal mechanisms that operate early after motor practice and mediate motor memory consolidation remain virtually unknown and require further studies [32].

2.4.4 Attentional control

The interaction in learning scores between MP and AC suggests that attention to visual elements and contextual cues of learning did not produce learning per se but affected learning outcomes at 24 hours (16% post-to-retention in AC, Fig. 2). Thus, the improvement in score at retention in AC must have occurred offline and was caused by a familiarization effect and/or cognitive processes. Because even after adjusting for learning due to familiarization with the motor task and repeated testing, there was still 1.5° less net error in AC compared with the control group, the possibility exists but requires further confirmation that the offline learning at retention in AC was related to procedural elements of the task. Processing of auditory, tactile, and visual information, as in the present study, can affect motor learning, as can cognitive processes such as attention to task details [6, 56]. Error-based learning engages the basal ganglia thalamocortical loops, medial cerebellum, the anterior cingulate cortex, the inferior frontal gyrus, and visual and parietal cortical areas, structures associated with cognitive aspects of the task, such as error detection and correction, working memory, and attention [6, 32, 57, 82]. More specifically, Thomson et al. (2008) reported that spatial attentional load but not variation in intensity of attention associated with dual tasking reduced SICI between successive responses of an index finger abduction task [91]. These results are in contrast to our data showing increase in SICI in AC (Fig. 4a, b). Thus, it remains unclear if recalling and anticipating the encoded visual cues associated with the motor task contributed to the improved performance at retention 24 hours after the learning bout in AC.

It is possible that subjects in AC imagined themselves making the movement required for the visuomotor task, although we gave no such instructions. In this regard, our results are in agreement with the findings of a previous study [92], reporting motor performance gains in young individuals as a result of motor imagery after sleep. This interpretation is complicated by data suggesting that the age-related decline in motor imagery is more severe in complex motor tasks and tasks in laboratory settings compared with simple motor tasks and real-life settings [93]. Furthermore, studies have shown decreased inhibition after motor imagery, similar to executing real movements [94, 95]. In our study, the task was complex and motor cortical inhibition increased in AC. It is therefore unlikely that the AC group imagined making the movement required for the task.

2.4.5 Limitations

Our design prevents us from drawing any inferences as to how motor performance, retention, and the neuronal mechanisms would compare with those in young adults. However, baseline differences between two age groups in motor performance complicated the interpretation of learning and retention data in numerous previous studies using the young-old comparison design [61]. Although we measured corticospinal excitability at rest, during the task, and during 20% contraction to assess adaptations in corticospinal excitability, taking one point on a nonlinear recruitment curve

poses limits to our data and restricts the scope of interpretation. Furthermore, we only measured the M_{max} at rest, which limits the interpretation of the corticospinal excitability data during the task. It is well established that fast motor learning involves not only M1, the only structure we probed, but also the networks that include the supplementary motor area, premotor cortices, and dorsolateral premotor cortex [82, 96, 97]. We did not quantify the effects of the two interventions on attention, but a previous motor learning study reported no effects on fatigue and attention [12]. We did not examine any potential adaptations at the spinal level, but considering recent data from TMS-conditioned H-reflex paradigms, it is unlikely that H-reflex and F-wave measurements could have provided a definitive answer [98, 99]. Finally, we acknowledge the limitation of performing a high number of comparisons, increasing the likelihood of type I error in some of our analyses.

2.5 Conclusions

We observed 40% motor learning after just 20 minutes of practice of a visuomotor task, a skill that naive healthy old adults were able to consolidate into motor memory 24 hours later. The skill, however, did not transfer to a task variant. In contrast, watching the same templates without actual movements produced no learning. Corticospinal excitability at rest and during the task did not change but strongly increased during 20% MVC in MP. Intracortical inhibition at rest and during the task decreased and facilitation at rest increased in MP. TMS metrics changed in the opposite direction in AC. The within-group changes and between-group differences were especially profound at retention administered 24 hours after the two interventions. Motor cortical disinhibition as inferred from changes in SICI measured in the active muscle emerged as key mechanisms mediating learning and motor memory consolidation. The present results collectively suggest that the healthily aging motor brain can learn and retain a complex motor skill but may have some difficulty in transferring the acquired skill to a task variant. The results may also have relevance for the rehabilitation of old adults' motor function compromised by neuronal injuries and disorders (e.g., stroke), requiring motor cortical reorganization through use-dependent plasticity.

References

1. Aagaard P, Suetta C, Caserotti P, Magnusson SP, Kjaer M. Role of the nervous system in sarcopenia and muscle atrophy with aging: Strength training as a countermeasure. Scand J Med Sci Sports. 2010;20(1):49-64.

2. Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. Neuroimage. 2001;14:21-36.

3. Goble DJ, Coxon JP, Wenderoth N, Van Impe A, Swinnen SP. Proprioceptive sensibility in the elderly: Degeneration, functional consequences and plastic-adaptive processes. Neurosci Biobehav Rev. 2009;33(3):271-8.

4. Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RS, Busa E, et al. Thinning of the cerebral cortex in aging. Cereb Cortex. 2004;14(7):721-30.

5. Ward NS, Frackowiak RS. Age-related changes in the neural correlates of motor performance. Brain. 2003;126(Pt 4):873-88.

6. Seidler RD. Neural correlates of motor learning, transfer of learning, and learning to learn. Exerc Sport Sci Rev. 2010;38(1):3-9.

7. Seidler RD, Bernard JA, Burutolu TB, Fling BW, Gordon MT, Gwin JT, et al. Motor control and aging: Links to age-related brain structural, functional, and biochemical effects. Neurosci Biobehav Rev. 2010;34(5):721-33.

8. Spirduso WW. Physical dimensions of aging. 2010.

9. Clark DJ, Fielding RA. Neuromuscular contributions to age-related weakness. J Gerontol A Biol Sci Med Sci. 2012;67(1):41-7.

10. Krakauer JW. Motor learning: Its relevance to stroke recovery and neurorehabilitation. Curr Opin Neurol. 2006;19(1):84-90.

11. Hummel FC, Steven B, Hoppe J, Heise K, Thomalla G, Cohen LG, et al. Deficient intracortical inhibition (SICI) during movement preparation after chronic stroke. Neurology. 2009;72(20):1766-72.

12. Zimerman M, Nitsch M, Giraux P, Gerloff C, Cohen LG, Hummel FC. Neuroenhancement of the aging brain: Restoring skill acquisition in old subjects. Ann Neurol. 2013;73(1):10-5.

13. Czaja SJ, Sharit J. The aging of the population: Opportunities and challenges for human factors engineering. The Bridge: Linking Engineering and Society. 2009;39(1):34-40.

14. Coats RO, Wilson AD, Snapp-Childs W, Fath AJ, Bingham GP. The 50s cliff: Perceptuo-motor learning rates across the lifespan. PLoS One. 2014;9(1):e85758.

15. Brown RM, Robertson EM, Press DZ. Sequence skill acquisition and off-line learning in normal aging. PLoS One. 2009;4(8):e6683.

16. Roig M, Ritterband-Rosenbaum A, Lundbye-Jensen J, Nielsen JB. Aging increases the susceptibility to motor memory interference and reduces off-line gains in motor skill learning. Neurobiol Aging. 2014;35(8):1892-1900.

17. Swinnen SP. Age-related deficits in motor learning and differences in feedback processing during the production of a bimanual coordination pattern. Cognitive Neuropsychology. 1998;15(5):439-66.

18. Cirillo J, Todd G, Semmler JG. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. Eur J Neurosci. 2011;34(11):1847-56.

19. Janacsek K, Nemeth D. Predicting the future: From implicit learning to consolidation. Int J Psychophysiol. 2012;83(2):213-21.

20. Nemeth D, Janacsek K, Londe Z, Ullman MT, Howard DV, Howard Jr JH. Sleep has no critical role in implicit motor sequence learning in young and old adults. Experimental brain research. 2010;201(2):351-8.

21. Nemeth D, Janacsek K. The dynamics of implicit skill consolidation in young and elderly adults. J Gerontol B Psychol Sci Soc Sci. 2011;66(1):15-22.

22. Rogasch NC, Dartnall TJ, Cirillo J, Nordstrom MA, Semmler JG. Corticomotor plasticity and learning of a ballistic thumb training task are diminished in older adults. J Appl Physiol (1985). 2009;107(6):1874-83.

23. Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, et al. Early consolidation in human primary motor cortex. Nature. 2002;415(6872):640-4.

24. Butefisch CM, Davis BC, Wise SP, Sawaki L, Kopylev L, Classen J, et al. Mechanisms of use-dependent plasticity in the human motor cortex. Proc Natl Acad Sci U S A. 2000;97(7):3661-5.

25. Sawaki L, Boroojerdi B, Kaelin-Lang A, Burstein AH, Butefisch CM, Kopylev L, et al. Cholinergic influences on use-dependent plasticity. J Neurophysiol. 2002;87(1):166-71.

26. Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, et al. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol. 1998;508(Pt 2):625-33.

27. Smith AE, Sale MV, Higgins RD, Wittert GA, Pitcher JB. Male human motor cortex stimulus-response characteristics are not altered by aging. J Appl Physiol (1985). 2011;110(1):206-12.

28. Di Lazzaro V, Oliviero A, Profice P, Saturno E, Pilato F, Insola A, et al. Comparison of descending volleys evoked by transcranial magnetic and electric stimulation in conscious humans. Electroencephalogr Clin Neurophysiol. 1998;109(5):397-401.

29. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. J Physiol. 1993;471:501-19.

30. Floyer-Lea A, Wylezinska M, Kincses T, Matthews PM. Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. J Neurophysiol. 2006;95(3):1639-44.

31. Cirillo J, Rogasch NC, Semmler JG. Hemispheric differences in use-dependent corticomotor plasticity in young and old adults. Exp Brain Res. 2010;205(1):57-68.

32. Dayan E, Cohen LG. Neuroplasticity subserving motor skill learning. Neuron. 2011;72(3):443-54.

33. Saucedo Marquez CM, Ceux T, Wenderoth N. Attentional demands of movement observation as tested by a dual task approach. PLoS One. 2011;6(11):e27292.

34. McNevin NH, Wulf G, Carlson C. Effects of attentional focus, self-control, and dyad training on motor learning: Implications for physical rehabilitation. Phys Ther. 2000;80(4):373-85.

35. Kempen GI, Miedema I, Ormel J, Molenaar W. The assessment of disability with the groningen activity restriction scale. conceptual framework and psychometric properties. Soc Sci Med. 1996;43(11):1601-10.

36. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.

37. Oldfield RC. The assessment and analysis of handedness: The edinburgh inventory. Neuropsychologia. 1971;9(1):97-113.

38. Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009;120(12):2008-39.

39. Kantak SS, Winstein CJ. Learning-performance distinction and memory processes for motor skills: A focused review and perspective. Behav Brain Res. 2012;228(1):219-31.

40. Buysse DJ, Reynolds CF,3rd, Monk TH, Berman SR, Kupfer DJ. The pittsburgh sleep quality index: A new instrument for psychiatric practice and research. Psychiatry Res. 1989;28(2):193-213.

41. Perez MA, Lungholt BK, Nyborg K, Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. Exp Brain Res. 2004;159(2):197-205.

42. Jensen JL, Marstrand PC, Nielsen JB. Motor skill training and strength training are associated with different plastic changes in the central nervous system. J Appl Physiol (1985). 2005;99(4):1558-68.

43. Tiffin J, Asher EJ. The purdue pegboard; norms and studies of reliability and validity. J Appl Psychol. 1948;32(3):234-47.

44. Thomas RM. Creating human development theories: A guide for the social sciences and humanities. ABC-CLIO; 2014.

45. Desrosiers J, Hebert R, Bravo G, Dutil E. The purdue pegboard test: Normative data for people aged 60 and over. Disabil Rehabil. 1995;17(5):217-24.

46. Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: Basic principles and procedures for routine clinical application. report of an IFCN committee. Electroencephalogr Clin Neurophysiol. 1994;91(2):79-92.

47. Kujirai K, Kujirai T, Sinkjaer T, Rothwell JC. Associative plasticity in human motor cortex during voluntary muscle contraction. J Neurophysiol. 2006;96(3):1337-46.

48. Rothwell JC, Hallett M, Berardelli A, Eisen A, Rossini P, Paulus W. Magnetic stimulation: Motor evoked potentials. the international federation of clinical neurophysiology. Electroencephalogr Clin Neurophysiol Suppl. 1999;52:97-103.

49. Barthelemy D, Alain S, Grey MJ, Nielsen JB, Bouyer LJ. Rapid changes in corticospinal excitability during force field adaptation of human walking. Exp Brain Res. 2012;217(1):99-115.

50. Forman D, Raj A, Button DC, Power KE. Corticospinal excitability of the biceps brachii is higher during arm cycling than an intensity-matched tonic contraction. J Neurophysiol. 2014;112(5):1142-51.

51. Petersen N, Christensen LO, Nielsen J. The effect of transcranial magnetic stimulation on the soleus H reflex during human walking. J Physiol. 1998;513(Pt 2):599-610.

52. Sidhu SK, Hoffman BW, Cresswell AG, Carroll TJ. Corticospinal contributions to lower limb muscle activity during cycling in humans. J Neurophysiol. 2012;107(1):306-14.

53. Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC. Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. J Physiol. 2008;586(Pt 21):5147-59.

54. Kojima S, Onishi H, Sugawara K, Kirimoto H, Suzuki M, Tamaki H. Modulation of the cortical silent period elicited by single- and paired-pulse transcranial magnetic stimulation. BMC Neurosci. 2013;14:43,2202-14-43.

55. Solnik S, DeVita P, Rider P, Long B, Hortobagyi T. Teager-kaiser operator improves the accuracy of EMG onset detection independent of signal-to-noise ratio. Acta Bioeng Biomech. 2008;10(2):65-8.

56. Wolpert DM, Diedrichsen J, Flanagan JR. Principles of sensorimotor learning. Nat Rev Neurosci. 2011;12(12):739-51.

57. Seidler RD, Noll DC. Neuroanatomical correlates of motor acquisition and motor transfer. J Neurophysiol. 2008;99(4):1836-45.

58. Luft AR, Buitrago MM. Stages of motor skill learning. Mol Neurobiol. 2005;32(3):205-16.

59. Rickard TC, Cai DJ, Rieth CA, Jones J, Ard MC. Sleep does not enhance motor sequence learning. Journal of Experimental Psychology: Learning, Memory, and Cognition. 2008;34(4):834.

60. Brawn TP, Fenn KM, Nusbaum HC, Margoliash D. Consolidating the effects of waking and sleep on motor-sequence learning. J Neurosci. 2010;30(42):13977-82.

61. Vallence AM, Goldsworthy MR. Can noninvasive brain stimulation enhance function in the ageing brain? J Neurophysiol. 2014;111(1):1-3.

62. Fathi D, Ueki Y, Mima T, Koganemaru S, Nagamine T, Tawfik A, et al. Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. Clin Neurophysiol. 2010;121(1):90-3.

63. Freitas C, Perez J, Knobel M, Tormos JM, Oberman L, Eldaief M, et al. Changes in cortical plasticity across the lifespan. Front Aging Neurosci. 2011;3:5.

64. Muller-Dahlhaus JF, Orekhov Y, Liu Y, Ziemann U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res. 2008;187(3):467-75.

65. Sawaki L, Yaseen Z, Kopylev L, Cohen LG. Age-dependent changes in the ability to encode a novel elementary motor memory. Ann Neurol. 2003;53(4):521-4.

66. Todd G, Kimber TE, Ridding MC, Semmler JG. Reduced motor cortex plasticity following inhibitory rTMS in older adults. Clin Neurophysiol. 2010;121(3):441-7.

67. Boyd LA, Vidoni ED, Siengsukon CF. Multidimensional motor sequence learning is impaired in older but not younger or middle-aged adults. Phys Ther. 2008;88(3):351-62.

68. Burke SN, Barnes CA. Neural plasticity in the ageing brain. Nature Reviews Neuroscience. 2006;7(1):30-40.

69. Coxon JP, Peat NM, Byblow WD. Primary motor cortex disinhibition during motor skill learning. J Neurophysiol. 2014;112(1):156-64.

70. Tunovic S, Press DZ, Robertson EM. A physiological signal that prevents motor skill improvements during consolidation. J Neurosci. 2014;34(15):5302-10.

71. Cheney PD, Fetz EE. Functional classes of primate corticomotoneuronal cells and their relation to active force. J Neurophysiol. 1980;44(4):773-91.

72. Muir RB, Lemon RN. Corticospinal neurons with a special role in precision grip. Brain Res. 1983;261(2):312-6.

73. Kernell D, Hultborn H. Synaptic effects on recruitment gain: A mechanism of importance for the input-output relations of motoneurone pools? Brain Res. 1990;507(1):176-9.

74. Castro-Alamancos MA, Donoghue JP, Connors BW. Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex. J Neurosci. 1995;15(7):5324-33.

75. Hess G, Donoghue JP. Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. J Neurophysiol. 1994;71(6):2543-7.

76. Stagg CJ, Bachtiar V, Johansen-Berg H. The role of GABA in human motor learning. Curr Biol. 2011;21(6):480-4.

77. Hinder MR, Schmidt MW, Garry MI, Carroll TJ, Summers JJ. Absence of cross-limb transfer of performance gains following ballistic motor practice in older adults. J Appl Physiol (1985). 2011;110(1):166-75.

78. Gallasch E, Christova M, Krenn M, Kossev A, Rafolt D. Changes in motor cortex excitability following training of a novel goal-directed motor task. Eur J Appl Physiol. 2009;105(1):47-54.

79. Garry MI, Kamen G, Nordstrom MA. Hemispheric differences in the relationship between corticomotor excitability changes following a fine-motor task and motor learning. J Neurophysiol. 2004;91(4):1570-8.

80. Liepert J, Classen J, Cohen LG, Hallett M. Task-dependent changes of intracortical inhibition. Exp Brain Res. 1998;118(3):421-6.

81. Rosenkranz K, Kacar A, Rothwell JC. Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. J Neurosci. 2007;27(44):12058-66.

82. Hikosaka O, Nakamura K, Sakai K, Nakahara H. Central mechanisms of motor skill learning. Curr Opin Neurobiol. 2002;12(2):217-22.

83. Chen R. Interactions between inhibitory and excitatory circuits in the human motor cortex. Exp Brain Res. 2004;154(1):1-10.

84. Dudai Y. The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol. 2004;55:51-86.

85. Fischer S, Nitschke MF, Melchert UH, Erdmann C, Born J. Motor memory consolidation in sleep shapes more effective neuronal representations. J Neurosci. 2005;25(49):11248-55.

86. Korman M, Raz N, Flash T, Karni A. Multiple shifts in the representation of a motor sequence during the acquisition of skilled performance. Proc Natl Acad Sci U S A. 2003;100(21):12492-7.

87. Robertson EM. From creation to consolidation: A novel framework for memory processing. PLoS Biol. 2009;7(1):e19.

88. Walker MP, Brakefield T, Hobson JA, Stickgold R. Dissociable stages of human memory consolidation and reconsolidation. Nature. 2003;425(6958):616-20.

89. Doyon J, Benali H. Reorganization and plasticity in the adult brain during learning of motor skills. Curr Opin Neurobiol. 2005;15(2):161-7.

90. Robertson EM, Pascual-Leone A, Miall RC. Current concepts in procedural consolidation. Nat Rev Neurosci. 2004;5(7):576-82.

91. Thomson RH, Garry MI, Summers JJ. Attentional influences on short-interval intracortical inhibition. Clin Neurophysiol. 2008;119(1):52-62.

92. Debarnot U, Creveaux T, Collet C, Doyon J, Guillot A. Sleep contribution to motor memory consolidation: A motor imagery study. Sleep. 2009;32(12):1559-65.

93. Kalicinski M, Kempe M, Bock O. Motor imagery: Effects of age, task complexity, and task setting. Exp Aging Res. 2015;41(1):25-38.

94. Kumru H, Soto O, Casanova J, Valls-Sole J. Motor cortex excitability changes during imagery of simple reaction time. Exp Brain Res. 2008;189(3):373-8.

95. Liepert J, Neveling N. Motor excitability during imagination and observation of foot dorsiflexions. J Neural Transm. 2009;116(12):1613-9.

96. Petersen SE, van Mier H, Fiez JA, Raichle ME. The effects of practice on the functional anatomy of task performance. Proc Natl Acad Sci U S A. 1998;95(3):853-60.

97. Sun FT, Miller LM, Rao AA, D'Esposito M. Functional connectivity of cortical networks involved in bimanual motor sequence learning. Cereb Cortex. 2007;17(5):1227-34.

98. Leukel C, Taube W, Rittweger J, Gollhofer A, Ducos M, Weber T, et al. Changes in corticospinal transmission following 8weeks of ankle joint immobilization. Clin Neurophysiol. 2015;126(1):131-9.

99. Taube W, Leukel C, Nielsen JB, Lundbye-Jensen J. Repetitive activation of the corticospinal pathway by means of rTMS may reduce the efficiency of corticomotoneuronal synapses. Cereb Cortex. 2014;25(6):1629-37.

I Chapter 3 I

Neuronal mechanisms of motor learning are age dependent

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Abstract

There is controversy whether age-related neuroanatomical and neurophysiological changes in the central nervous system affect healthy old adults' abilities to acquire and retain motor skills. We examined the effects of age on motor skill acquisition and retention and potential underlying mechanisms by measuring corticospinal and intracortical excitability, using transcranial magnetic stimulation. Healthy young (n = 24, 22 years) and old (n = 22, 71 years) adults practiced a wrist flexion-extention visuomotor task or only watched the templates as an attentional control for 20 minutes. Old compared with young adults performed less well at baseline. Although the absolute magnitude of skill acquisition and retention was similar in the 2 age groups (age × intervention × time, $p = 0.425$), a comparison of baseline-similar age sub-groups revealed impaired skill acquisition but not retention in old versus young. Furthermore, the neuronal mechanisms differed as revealed by an opposite direction of associations in the age-groups between relative skill acquisition and intracortical facilitation during the task, and opposite changes during skill retention in corticospinal excitability at rest and during the task and intracortical inhibition during the task.

3.1 Introduction

For an enjoyable daily life, children, adults, and seniors need to acquire new motor skills and retain previously acquired abilities. Motor skill acquisition and the need to be able to perform previously learned skills relatively free of error are particularly relevant for the increasing number of old adults [1]. Beyond gross motor skills, old adults must also cope with new technologies that require manipulative motor challenges, such as operating computer keyboards and portable electronic devices that are reconfigured with each upgrade.

It is expected that old adults' abilities to acquire unfamiliar motor skills would decline based on the numerous and predominantly unfavorable age-related neuroanatomical and neurophysiological changes [2-4]. However, it is actually unclear whether and to what extent advancing age impairs skill acquisition. Although some studies suggest that motor skill acquisition is impaired, [5-8], other studies show similar [9] or even superior [10] capacity to acquire new motor skills in old as compared to young adults. One of the reasons for these inconsistencies is that baseline motor performance levels are similar [11] or different [10] between age groups.

In addition to motor skill acquisition, it is equally unclear to what extent age affects motor skill retention. One study reported that old adults only stabilize motor performance after a 24-hour offline period of no training, whereas young adults are able to further increase skill performance beyond levels of stabilization [10]. In other experiments, the improvements in performance after the 12-hour offline period are smaller in old adults compared with young adults, and young adults further increase performance until a week after training, whereas old adults did not [12].

With much inconsistency concerning the effects of age on the magnitude of motor skill acquisition and retention, it is not unexpected that there is also disagreement on the possible mechanisms underlying these processes. For example, diffusion tensor imaging and functional magnetic resonance imaging (fMRI) studies showed contradictory results regarding neuronal mechanisms of motor skill acquisition in aging [13-16]. On the other hand, transcranial magnetic stimulation (TMS) studies revealed consistently no effects of age but inconsistent results regarding the effect of motor practice on TMS variables. Regardless of age, corticospinal excitability (CSE), measured as the peak-to-peak amplitude of the motor-evoked potential (MEP), increased during motor skill acquisition [8 - left thumb, 9] or did not change [8 - right thumb]. In contrast, 1 study showed age-related differences in CSE after 10 minutes of motor practice [11]. CSE increased in young but remained unchanged in old adults. In addition to CSE, age did not either affect changes in short-interval intracortical inhibition (SICI) during motor skill acquisition, although the directions of change are different between studies, showing decreases [9] or no changes [8, 11].

The underlying neuronal mechanisms of motor skill retention in aging remain unclear. Only 1 fMRI study has examined age-related changes in neuronal networks during skill retention, showing clear age-related differences in brain connectivity [17]. Three days after interleaved practice of a motor sequence, functional connectivity increased in old adults between the right and left dorsolateral prefrontal cortex (DLPFC) and between the dorsal premotor cortex and inferior parietal cortex. However, the functional connectivity in young adults increased between DLPFC and the supplementary motor area and inferior frontal gyrus. To the best of our knowledge, no

study has yet examined changes in potential neuronal mechanisms of motor skill retention in young and old adults using TMS.

In an effort to address the many inconsistencies, we examined the effects of age on motor skill acquisition and retention as well as potential underlying mechanisms by measuring corticospinal and motor cortical excitability using TMS in both young and old adults. We paid particular attention to baseline differences in motor skills between the 2 age groups [18] by using multilevel analyses. Based on previous studies, we expected that (1) old adults compared with young adults would perform less well at baseline on the visuomotor task [9]; (2) both age groups would improve motor performance similarly relative to baseline [9]; (3) old adults would improve their motor performance less than young adults during the 24-hour offline period; and (4) there would be no age-related differences in practice-related changes in motor cortical and corticospinal function. Furthermore, as attentional resources are known to be involved in motor learning [19, 20], we controlled for attentional load of the motor practice. Because attention activates brain areas similar to those used in motor skill acquisition [17, 19, 21] and aging is associated with a decline in attention [22], we expected that (5) old versus young adults in the attentional control group would improve motor performance to a lesser extent.

3.2 Methods

3.2.1. Participants

Twenty-four young adults (18 – 30 years, 12 male) participated in the main experiment, and the data of these young adults are compared with the data of the 22 old adults (≥ 65 years, 14 male) who participated in our previous study [23]. In addition to the main experiment, 12 young and 5 old adults participated in a control experiment. All participants were right-handed [24]. All participants signed an informed consent document before participating in a study protocol that was approved by the Medical Ethical Committee of the University Medical Center Groningen.

3.2.2 General organization of the study

The young adults performed the same testing procedures and training protocol as the old adults did and as described detailed previously [23], with the only exception that the Mini Mental State Examination and Groningen Activity Restriction Scale questionnaires were not assessed in young adults. Fig. 1 shows the study design. In summary, participants practiced a wrist flexion-extension visuomotor task, in which they had to match a preprogrammed template as accurately as possible (motor practice group; MP) or only watched the templates for 20 minutes to control for attentional demands (attentional control group; AC). TMS was performed with the right extensor carpi radialis as the target muscle.

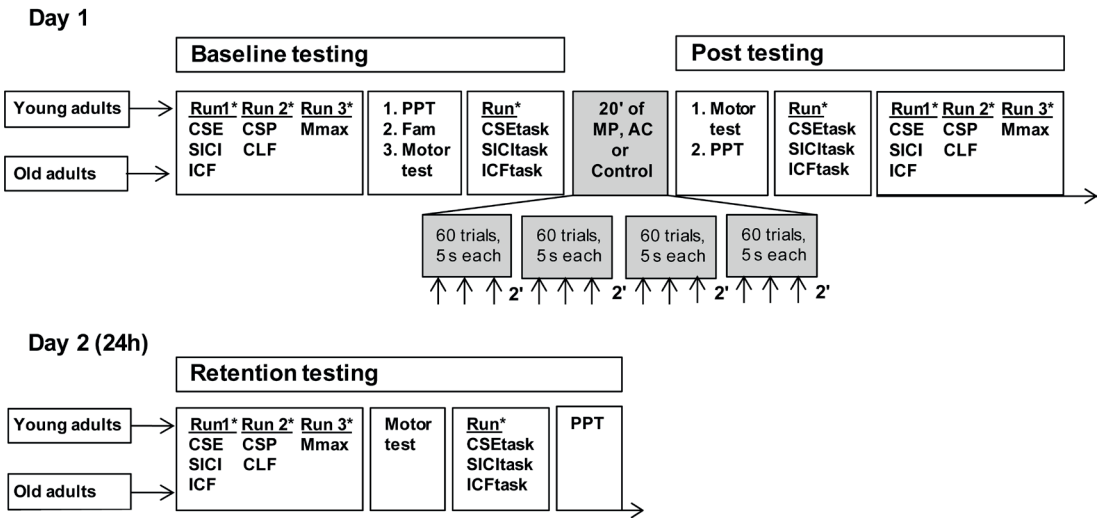

Fig. 1 Young and old adults followed the same experimental design. Day 1 consisted of a baseline test, an intervention, and posttest, and Day 2 consisted of a retention test. Upward directed arrows indicate the time when participants performed a counting task to control for attentional drift. The order of the runs within a block and the order of the pulses within a block were randomized (asterisk). Abbreviations: AC, attentional control; CLF, contralateral facilitation; CSE, corticospinal excitability, CSE_{task}, corticospinal excitability during task; CSP, cortical silent period; Fam, familiarization; ICF, intracortical facilitation; ICF_{task}, intracortical facilitation during task; M_{max}, maximal compound action potential; MP, motor practice; PPT, Purdue Pegboard test; SICI, short-interval intracortical inhibition; $SICI_{task}$ short-interval intracortical inhibition during task.

3.2.3 Statistical analysis

Data were tested for normality with the Shapiro–Wilk test. Non-normally distributed data were log transformed for analyses, but the nontransformed data are reported. Multivariate analyses of variances (MANOVA) were used to determine if participant characteristics differed between young and old adults. Repeated measures ANOVAs with within-subjects factor time (baseline, after intervention, and retention) and between-subject factor intervention (MP, AC) and age (young, old) were used to determine if young and old adults responded differently to the interventions on the dependent variables motor performance (°); maximal compound action potential (M_{max}, mV); CSE at rest (CSE_{rest}, $\%M_{max}$), during the task (CSE_{task}, $\%M_{max}$), and during an isometric contraction ($CSE_{contraction}$, $\%MEP_{rest}$); SICI at rest ($SICI_{rest}$,$\%MEP_{rest}$), and during the task ($SICI_{task}$, $\%MEP_{task}$); intracortical facilitation (ICF) at rest (ICF_{rest}, $\%MEP_{rest}$), and during the task (ICF_{task}, $\%MEP_{task}$); cortical silent period (CSP, ms). If the assumption of sphericity was violated, the Greenhouse-Geisser correction was used. In case of significant main or interaction effects, Tukey's post-hoc analysis was used to determine which groups differed at $p < 0.05$. Because nonsignificant interactions do not allow us to examine baseline differences between age groups, independent t-tests with grouping variable age were performed in this case. Furthermore, independent t-tests were used to examine whether relative changes in motor performance differed between $young_{MP}$ versus old_{MP} and between $young_{AC}$ versus old_{AC}.

Because there is evidence that motor performance in young versus old adults is higher [6, 9, 10] and baseline differences complicate comparisons of motor learning between age groups [18], we performed a secondary analysis using multilevel analysis (MLwin, version 2.29) for young and old participants assigned to the MP intervention only. Multilevel analysis can handle differences at baseline by allowing intercepts to vary between participants. Therefore, it allowed us to compare learning between the 2 age groups while accounting for variations at baseline. To that purpose, a random intercept and slope model (model 1) was constructed for motor performance in which time of measurement (level 1) was nested within participant (level 2). Subsequently, main effects of time (baseline, after intervention, retention) and age (young, old) were added to the model (model 2) to determine if the main effects were significant. Finally, an age × time interaction was added to the model (model 3) to address the main purpose of the multilevel analysis: to determine if motor skill acquisition and retention differed between young and old adults, had they started at the same baseline level.

A tertiary analysis ranked old and young adults based on motor performance at baseline. A selection of the 8 old adults with the smallest errors and the 8 young adults with the highest errors produced 2 groups that were nearly numerically identical in motor performance at baseline. We then reanalyzed the behavioral and TMS data in these subgroups.

To determine if baseline values and changes in motor performance were associated with baseline values and changes in neuronal measures, Spearman's rho correlation coefficients were computed between baseline values and absolute and percent change from baseline to after the intervention, absolute and percent change from after the intervention to retention, and absolute and percent change from baseline to retention of motor performance and TMS variables in $young_{MP}$ and old_{MP}. A nonparametric correlation analysis was chosen because not all variables were normally distributed and to preserve uniformity between correlation analyses. Furthermore, to determine if baseline values or changes in TMS variables were associated with baseline values or changes in other TMS variables, Spearman's rho correlations were computed across baseline values of TMS variables and across relative changes in all TMS variables.

For the control experiment, a repeated measures ANOVA using time as a within subjects factor was conducted to determine if there was a main effect of time in the young and old group on motor performance, Purdue Pegboard performance, M_{max} and the TMS variables. For all statistical analyses, the level of significance was set at $p < 0.05$.

3.3 Results

Table 1 shows the participant characteristics. Although old versus young adults were somewhat heavier, more right-handed, and reported somewhat less sleep the night before the retention test (age main effects, $p < 0.05$), the groups were similar for the majority of the characteristics.

3.3.1 Absolute motor learning is similar in old and young adults

Fig. 2 shows the motor learning data. At baseline, old versus young adults had 25% more error (old_{MP+AC}: 18.87 ± 3.72°; $young_{MP+AC}$: 14.12 ± 3.13°; $t_{44} = 4.7$, $p < 0.001$). Overall, old versus young

adults executed the visuomotor task with 33% more error (age main effect: $F_{1, 42} = 44.0$, $p < 0.001$). Old and young adults acquired and retained the skill at similar rates in MP and AC (age × time interaction: $F_{2, 84} = 1.9$, $p = 0.160$ and age × intervention × time interaction: $F_{2, 84} = 0.9$, $p = 0.425$). This observation was confirmed by the secondary, multilevel analysis outcome, showing no age × time interaction ($p = 0.283$ and 0.434 for skill acquisition and retention, respectively). However, relative to baseline, the magnitude of skill acquisition was lower in old_{MP} versus $young_{MP}$ ($t_{21} = 4.5$, $p < 0.001$). Table 2 summarizes the absolute and relative changes in motor performance. For all analyses, main effects of time and intervention, and age × intervention and intervention × time interactions are not reported because these effects are not required for testing the hypotheses.

Table 1. Characteristics of old and young adults in the motor practice and attentional control group.

Variable	Old		Young	
	MP, n = 11	AC, n = 11	MP, n = 12	AC, n = 12
Age (y)	71.3 (3.4)	70.5 (2.5)	22.7 (3.2)	22.2 (2.1)
Gender (M / F)	7 / 4	7 / 4	6 / 6	6 / 6
Height (m)	1.71 (0.10)	1.77 (0.07)	1.77 (0.09)	1.78 (0.08)
Mass (kg)	73.3 (9.3)	84.5 (18.3)	72.0 (15.1)	71.3 (7.6)
BMI (kg/m^2)[a]	24.9 (1.9)	27.4 (7.2)	22.8 (3.3)	22.4 (1.8)
Laterality quotient[a]	0.91 (0.09)	0.96 (0.08)	0.82 (0.19)	0.90 (0.15)
PSQI	5.2 (4.3)	5.0 (4.0)	4.4 (2.3)	4.8 (1.9)
Quantity of sleep (h)[a]	6.7 (1.7)	7.2 (0.9)	7.8 (1.0)	7.8 (1.0)
Quality of sleep[b]	1	1	1	1
M_{max} (mV)[c]	2.40 (0.76)	2.22 (0.76)	2.46 (1.39)	2.49 (1.35)
RMT (%SO)[c]	55.3 (12.8)	51.7 (10.8)	47.8 (6.1)	47.2 (7.3)
AMT (%SO)[c]	49.1 (14.8)	47.3 (5.4)	42.4 (6.5)	43.8 (6.7)

Values are mean (± standard deviation).

Abbreviations: AC, attentional control group; AMT, active motor threshold; BMI, body mass index; M_{max}, maximal compound action potential; MP, motor practice group; PSQI, Pittsburgh Sleep Quality Index (lower score is higher quality of sleep in last month); RMT, resting motor threshold; SO, stimulator output.

[a] Old versus young adults, $p < 0.05$.

[b] Median instead of mean, 4-point Likert scale, with a value of 0 and 3, respectively, denoting high and poor quality of sleep in the night before retention testing.

[c] Values are mean across the 3 test moments.

3.3.2 Hand function data

Old_{MP+AC} versus $young_{MP+AC}$ placed 19% or 3.3 pins less in the Purdue Pegboard at baseline and overall ($t_{44} = 7.17$, $p < 0.001$; age main effect $F_{1, 42} = 59.8$, $p < 0.001$). Old improved 6.2% or 0.8 pins (old_{MP}: 2.1% or 0.3 pins; old_{AC}: 10.3% or 1.4 pins) and young 7.7% or 1.3 pins ($young_{MP}$: 9.0% or 1.5 pins; $young_{AC}$: 6.4% or 1.1 pins; age × time interaction: $F_{2, 84} = 3.9$, $p = 0.024$; age × intervention × time interaction: $F_{2, 84} = 4.3$, $p = 0.017$).

3.3.3 Peripheral nerve stimulation data

At baseline, old_{MP+AC} and $young_{MP+AC}$ had similar peak-to-peak amplitudes of M_{max} ($p = 0.489$). There was no significant age main effect ($p = 0.754$), and there were no significant age × time or age × intervention × time interactions ($p = 0.142$ and 0.358, respectively).

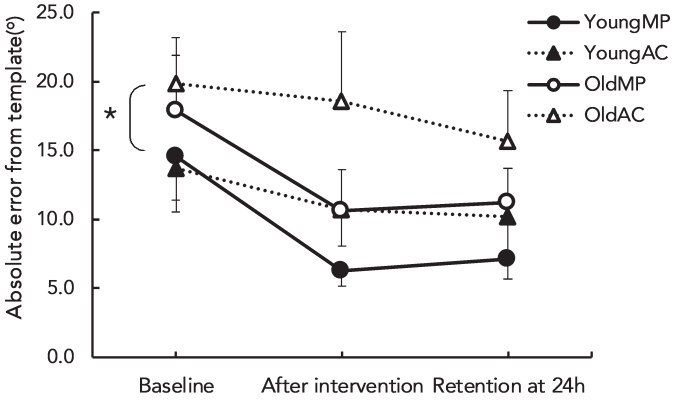

Fig. 2 Motor performance of old (open symbols) and young adults (filled symbols) at baseline, after motor practice (circles), or attentional control (triangles) and at retention. *Young$_{MP+AC}$ performed significantly better at baseline and averaged across all test-moments than Old$_{MP+AC}$ ($t_{44} = 4.7$, $p < 0.001$ and main effect of age: $F_{1, 42} = 44.0$, $p < 0.001$). Abbreviations: AC, attentional control; MP, motor practice.

Table 2. Summary of behavioral data.

Group	Baseline, °	Posttest, °	Retention, °	Change (°)[a]		Change (%)[a]	
				Baseline – posttest	Posttest – retention test	Baseline – posttest	Posttest – retention test
Old							
MP	17.91[b] (3.98)	10.64 (2.94)	11.23 (2.46)	-7.27	0.59	-40.5[c]	8.9
AC	19.83[b] (3.35)	18.54 (5.03)	15.65 (3.64)	-1.28	-2.89	-6.5	-13.2
Young							
MP	14.51 (3.12)	6.25 (1.12)	7.11 (1.45)	-8.25	0.86	-56.3	15.0
AC	13.73 (3.22)	10.69 (2.64)	10.15 (2.51)	-3.04	-0.55	-19.2	-2.1

Values are mean (± standard deviation).

[a] Negative change (%) means an improvement in performance.

[b] Old_{MP+AC} higher than $Young_{MP+AC}$ ($t_{44} = 4.7$, $p < 0.001$).

[c] $p < 0.05$ in comparison with $Young_{MP}$ ($t_{21} = 3.370$, $p = 0.003$).

3.3.4 Brain stimulation data

3.3.4.1 Corticospinal excitability

Fig. 3 shows representative examples of CSE at rest in an old and a young adult in MP. Fig. 4A shows the CSE group data at rest, measured as the mean MEP peak-to-peak amplitude, and Fig. 4B during the task in old and young adults. At baseline, there was no difference in CSE_{rest} between old_{MP+AC} and $young_{MP+AC}$ ($p = 0.677$). There was a significant age × intervention × time interaction ($F_{1.675, 70.363} = 3.5$, $p = 0.034$). At baseline, CSE_{rest} was higher in old_{MP} (15.5 ± 11.4 $\%M_{max}$) versus $young_{MP}$ (12.6 ± 9.1 $\%M_{max}$, $p < 0.05$). CSE_{rest} decreased by 25% from baseline to retention in old_{MP},

whereas it increased by 40% in $young_{MP}$, resulting in a 34% lower CSE_{rest} in old versus young adults at retention ($p < 0.05$). In addition, the difference in CSE_{rest} decrease from baseline to retention in old_{AC} versus $young_{AC}$ was significant ($p < 0.05$) but small (1.3% M_{max} vs. 2.7% M_{max}, respectively).

CSE_{task} was similar in old_{MP+AC} and $young_{MP+AC}$ at baseline ($p = 0.873$). There was an age × time interaction ($F_{2, 84} = 4.1$, $p = 0.019$) and an age × intervention × time interaction ($F_{2, 84} = 3.7$, $p = 0.030$). Directly after the intervention, CSE_{task} did not change in old_{MP} whereas it decreased in $young_{MP}$ by 22% relative to baseline. At retention, CSE_{task} decreased relative to directly after the intervention by 27% in old_{MP} whereas it increased by 52% in $young_{MP}$. In contrast to the effects in MP, there were no age-related differences in CSE_{task} in AC ($p > 0.05$).

At baseline, CSE measured during an isometric contraction at 20% MVC was lower in old_{MP+AC} (440.1 ± 316.2 $\%MEP_{rest}$) compared with $young_{MP+AC}$ (645.8 ± 436.2 $\%MEP_{rest}$; $t_{44} = 2.8$, $p = 0.008$). Averaged across both intervention groups and all test points, the value of $CSE_{contraction}$ was 106 $\%MEP_{rest}$ lower in old versus young adults (age main effect $F_{1, 42} = 6.7$, $p = 0.013$). Furthermore, there was a trend for an age × intervention × time interaction ($F_{2, 84} = 3.0$, $p = 0.058$).

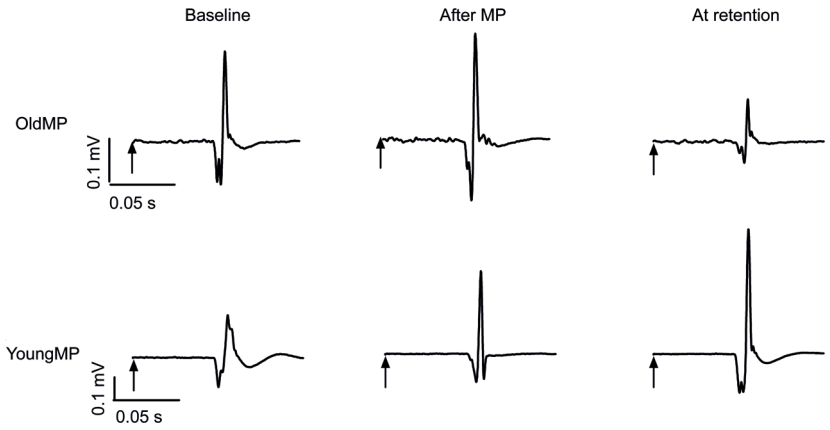

Fig. 3 Representative responses to transcranial magnetic stimulation in the right extensor carpi radialis muscle of 1 old (72 years) and 1 young (23 years) adult at baseline, after motor practice (MP), and at retention. Waveforms represent average of 10 motor-evoked potentials in response to a single test pulse at 120% resting motor threshold. Arrows indicate when the test pulse is given. Note the difference in length of the vertical calibration bars.

3.3.4.2 Short-interval intracortical inhibition

At baseline, old_{MP+AC} and $young_{MP+AC}$ had similar SICI values measured at rest ($p = 0.379$) and during the task ($p = 0.898$). No main or interaction effects occurred for SICI measured at rest (all effects $p > 0.05$). Fig. 4C shows the age × intervention × time interaction for SICI measured during the task ($F_{2, 84} = 4.1$, $p = 0.021$). Directly after motor practice and at retention, there was a significant difference in SICI measured during the task between old_{MP} and $young_{MP}$. Inhibition did not change from baseline to after motor practice in old adults but decreased from $89\%MEP_{task}$ to $100\%MEP_{task}$ in young adults. For the same measurement, the direction of change was opposite at

retention: Inhibition decreased to 100%MEP_{task} in old_{MP} and it increased in $young_{MP}$ to 84%MEP_{task}. This resulted in respectively 13% and -16% between age-group differences after motor practice and at retention. There were no significant differences in $SICI_{task}$ between old_{AC} and $young_{AC}$ ($p > 0.05$).

3.3.4.3 Cortical silent period

Old_{MP+AC} had a shorter cortical silent period (71.5 ± 15.1 ms) than $young_{MP+AC}$ (84.6 ± 20.9 ms) at baseline ($t_{44} = 2.4$, $p = 0.021$) and averaged across all test moments (age main effect: $F_{1, 42} = 495.5$, $p < 0.001$).

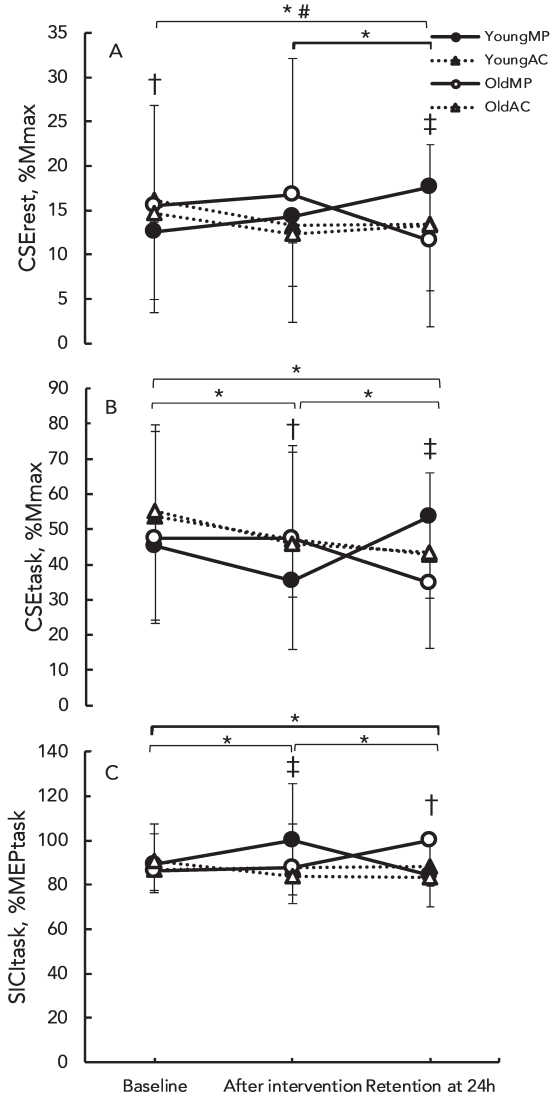

Fig. 4 CSE at rest (A) and during task performance (B), and short-interval intracortical inhibition during task performance (C) in young (filled symbols) and old (open symbols) adults at baseline, after motor practice (circles) or attentional control (triangles), and at retention. There was a significant age × intervention × time interaction for CSE at rest ($F_{1.675, 70.363} = 3.5$, $p = 0.034$) and during task performance ($F_{2, 84} = 3.7$, $p = 0.030$). There was also a significant age × intervention × time interaction for short-interval intracortical inhibition measured during the task ($F_{2, 84} = 4.1$, $p = 0.021$). *Change in $young_{MP}$ different from old_{MP}, #change in $young_{AC}$ different from old_{AC}, †$young_{MP}$ lower than old_{MP}, ‡$young_{MP}$ higher than oldMP. Abbreviations: AC, attentional control; CSE, corticospinal excitability; MEP, motor-evoked potential; M_{max}, maximal compound action potential; MP, motor practice; SICI, short-interval intracortical inhibition.

3.3.4.4 Intracortical facilitation

At baseline, there were no significant differences between old_{MP+AC} and $young_{MP+AC}$ in ICF measured at rest and during the task (p = 0.720 and 0.103, respectively). No main or interaction effects occurred for ICF at rest and during task performance (all p > 0.05), but the age × intervention × time interaction for ICF at rest approached significance ($F_{2, 84}$ = 2.9, p = 0.060).

3.3.5 Correlation analyses

Here, we report only the Spearman's rho correlations between baseline values of motor performance and baseline values of TMS variables and correlations between changes in motor performance and changes in TMS variables. Correlations between baseline values of TMS variables and between percent changes in TMS variables are reported in the Supplementary materials. Baseline motor performance correlated with baseline CSE at rest in old_{MP} (r_{s9} = 0.68, p = 0.022). Baseline motor performance did neither correlate with any other baseline values nor with any percent changes from baseline to after motor practice and from posttest to retention in motor performance and TMS variables in either age group (all p > 0.05).

In 4 of the 6 correlations between skill acquisition and changes in TMS outcomes, the changes in TMS metrics were not significant but the correlations between the pair wise changes were nonetheless statistically significant. Relative motor skill acquisition did not correlate with percent changes in CSE_{rest} after motor practice in old adults (r_{s9} = 0.19, p = 0.582) but negatively correlated in young adults (r_{s10} = -0.69, p = 0.013) (Fig. 5A), indicating that greater relative skill acquisition correlated with increases in CSE_{rest} in $young_{MP}$ but not in old_{MP}. However, when motor skill acquisition was measured in absolute units, these variables were not correlated (all p > 0.05).

Relative motor skill acquisition correlated with percent changes in SICI measured at rest in old_{MP} (r_{s9} = 0.77, p = 0.006) but not in $young_{MP}$ (r_{s10} = 0.14, p = 0.665) (Fig. 5B). However, absolute motor skill acquisition was not correlated with changes in $SICI_{rest}$ in both age groups (p > 0.05). Both absolute and relative motor learning did not correlate with changes in $SICI_{rest}$ from baseline to retention in either age group (all p > 0.05). In contrast with changes in SICI values at rest, there was a trend for an inversed relationship between percent changes in SICI during the task and relative motor skill acquisition in old_{MP} (r_{s9} = -0.55, p = 0.083; Fig. 5C) but a trend toward a positive correlation for $young_{MP}$ (r_{s10} = 0.52, p = 0.080; Fig. 5C). When motor skill acquisition was measured in absolute units, it was not correlated with changes in $SICI_{task}$ in both age groups (both p-values > 0.05). Changes in the duration of the contralateral silent period from after the intervention to retention were inversely correlated with both absolute and relative motor skill retention in old_{MP} (r_{s9} = -0.63, p = 0.039 and r_{s9} = -0.66, p = 0.026, respectively; Fig. 5D shows correlation for relative skill retention). In sum, (1) whether motor skill acquisition correlated with percent changes in $SICI_{rest}$ and $SICI_{task}$ depends on age and whether motor skill acquisition is measured in absolute or relative units and (2) absolute and relative increases in motor performance from posttest to retention correlated with longer silent periods in old but not in young adults.

There was an opposite correlation in old and young adults between relative motor skill acquisition and the pre/post changes in ICF_{task} (old_{MP}: r_{s9} = -0.62, p = 0.043; $young_{MP}$: r_{s10} = 0.76, p = 0.004;

Fig. 5E). Using absolute motor skill acquisition, this relationship also was in opposite direction for old and young adults but was not significant for old adults (old_{MP}: r_{s9} = -0.49, p = 0.125; $young_{MP}$: r_{s10} = 0.62, p = 0.033). Finally, there was a trend for a negative correlation in $young_{MP}$ between absolute and relative changes in motor performance and changes in ICF during the task from posttest to retention (r_{s10} = -0.53, p = 0.075, r_{s10} = -0.57, p = 0.055, respectively). This indicates that greater absolute and relative skill acquisition tended to be associated with, respectively, an increase and a decrease in ICF during the task in old and young adults, and greater absolute and relative retention of skill in young adults tended to be related to more facilitation. All remaining changes in neuronal mechanisms did not correlate with motor skill acquisition or retention (all p > 0.05).

3.3.6 Tertiary analyses

Fig. 6A shows that skill acquisition was 2.6° or 16.5% less in baseline-similar old_{MP} (n = 8, 16.1° error) compared with $young_{MP}$ (n = 8, 16.0° error; age × time interaction: $F_{2, 28}$ = 5.6, p = 0.009), in contrast to the main analysis. The pattern of skill retention was similar in this tertiary and the main analysis, showing no age-related differences. There was a complete agreement between the main (11 old, 12 young) and the tertiary analysis (8 old, 8 young) with respect to the trends and direction in the TMS data. To illustrate, Fig. 6B-D show that the changes in CSE_{rest}, CSE_{task} and $SICI_{task}$ were similar in the tertiary and the main analyses (Fig. 4).

3.3.7 Control experiment

Old adults in the control group marginally improved their performance on the visuomotor task (2.7° increase from baseline to retention, p = 0.056) but the TMS variables, M_{max} and Purdue Pegboard performance did not change (all p > 0.05). Young control participants increased their motor performance by 4.3° from baseline to posttest and 0.8° from posttest to retention on Day 2 (time main effect: $F_{2, 22}$ = 29.8, p < 0.001). In addition, Purdue Pegboard performance increased slightly in this group (baseline: 16.8 ± 1.5, posttest: 17.5 ± 1.4, retention: 17.6 ± 1.4; time main effect: $F_{2, 22}$ = 5.3, p = 0.013). M_{max} and TMS variables were stable over time in the young control group (all p > 0.05).

3.4 Discussion

We examined the effects of age on motor skill acquisition and retention and potential underlying mechanisms by measuring corticospinal and motor cortical excitability using TMS. We found that, although old adults performed less well than young adults on the visuomotor task at each of the 3 measurement points, age did not affect skill acquisition and retention when these behavioral changes were expressed in absolute units. However, age does seem to affect skill acquisition (but not skill retention) when a subgroup of old and young subjects start at the same baseline level, and age also seems to affect the neuronal mechanisms we probed in relation to skill acquisition and retention of a visuomotor skill.

3.4.1 Motor performance is lower in old versus young adults

Overall, old versus young adults performed less well on both the visuomotor task and the Purdue Pegboard test. Such age-related declines in motor performance agree with previous studies [6,

9, 10] and are generally attributed to the age-related changes in the central nervous system and neuromuscular system (for a review see [3]). Despite the well-characterized dysfunctions in the aging neuronal and muscular systems, there are several studies that do not report negative effects of age on motor performance [5, 8, 11, 25].

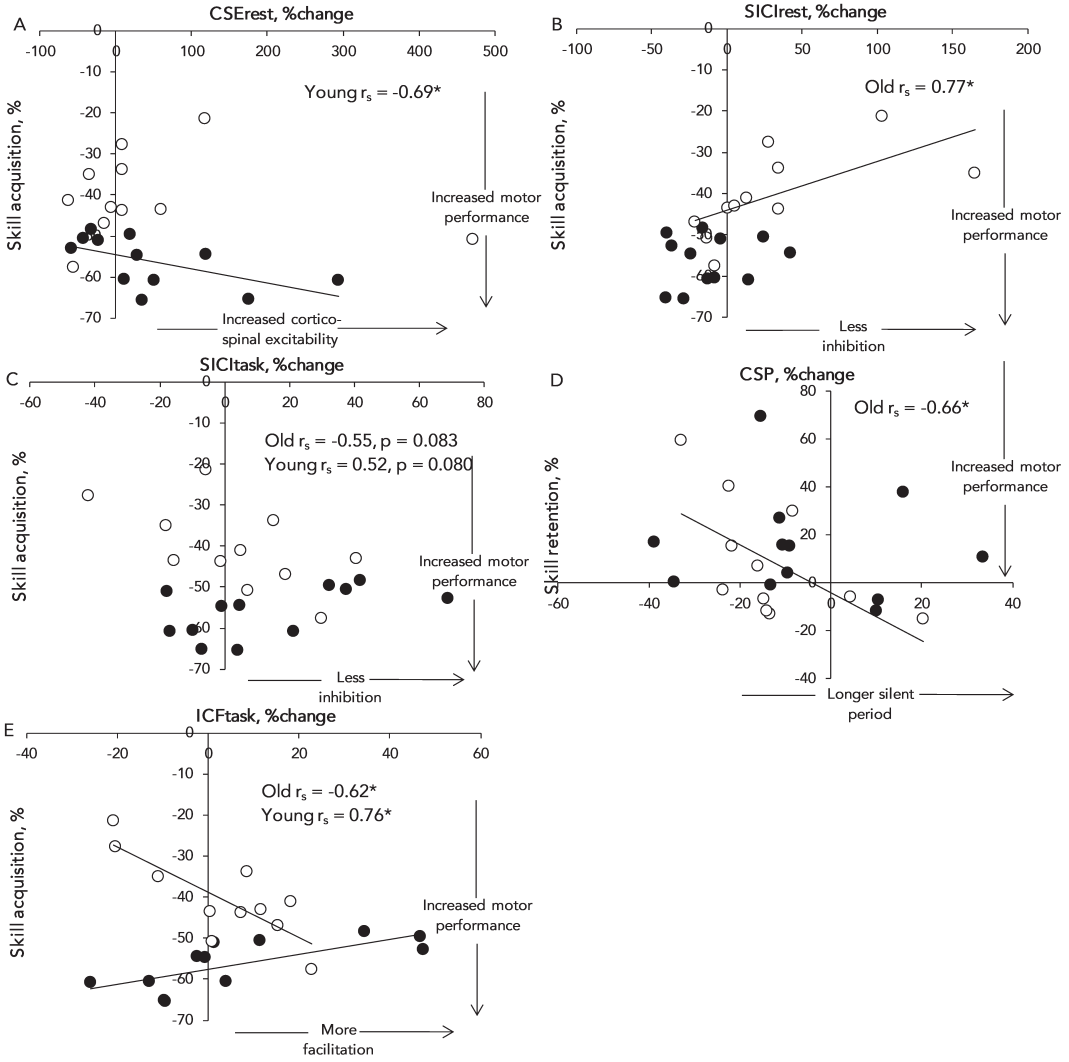

Fig. 5 Spearman's rho correlations between percent changes in motor performance and percent changes in (A) CSE measured at rest (young: R^2 = 0.33, y = -0.03x - 54.6); (B) short-interval intracortical inhibition measured at rest (old: R^2 = 0.41, y = 0.12x - 44.2) and (C) during the task; (D) contralateral silent period (old: R^2 = 0.35, y = -1.01x – 4.0); (E) intracortical facilitation measured during the task (young: R^2 = 0.51, y = 0.18x - 57.6; old: R^2 = 0.64, y = -0.56x - 38.8). Graphs (A)-(C), (E) represent changes from baseline to after the motor practice, graph (D) represents changes from directly after the intervention to retention. Filled symbols represent young and open symbols represent old adults. Lines represent linear fit for significant correlations. Note that the figures show the data for the motor practice (MP) but not for the attentional control (AC) group. * $p < 0.05$. Abbreviations: CSE, corticospinal excitability; CSP, cortical silent period; ICF, intracortical facilitation; SICI, short-interval intracortical inhibition.

3.4.2 Effects of age on motor skill acquisition: absolute and relative gains

There is much inconsistency in the literature as to how, or whether, age affects healthy old adults' ability to acquire a novel motor skill. Conclusions concerning the effects of age on motor practice outcome seem to depend on whether the improvements are expressed in absolute or relative units. Our results showed similar absolute magnitude of motor skill acquisition in old and young adults, in agreement with a recent study examining skill acquisition on a bimanual coordination task [25]. In contrast, similar to multiple studies [5, 8, 11], our results show that old adults cannot improve their performance as much as young adults when measured relative to baseline. Skill acquisition measured in relative units depends on baseline differences between groups. Baseline differences observed in some [6, 9, 10] but not all studies [5, 8, 11] complicate group comparisons [18]. In the present study, we used a new approach and controlled for baseline differences by taking variations at baseline into account using a multilevel analysis, showing no interaction between age-group and time. Most of the previous studies did not control for baseline differences, and report either absolute or relative skill acquisition, which makes between-study comparisons difficult. Vallence and Goldsworthy suggested modifying task difficulty so that baseline levels do not differ between groups [18]. In the present study, old compared with young adults performed worse at baseline. However, in contrast to the main analysis, a tertiary analysis of baseline-similar age groups revealed that skill acquisition is impaired in old compared with young adults (compare Figs. 2 and 4 to Fig. 6), confirming previous data [5, 8, 11]. Future studies should report skill acquisition and retention in both absolute and relative units, and should discuss how differences between groups in motor skill at baseline affect the interpretation of the data. Besides differences in reporting the data, methodological differences in the type, complexity, and duration of the motor tasks and the joints involved in the tasks obviously also affect discordance in the effects of age on motor skill acquisition.

Against our expectations [10, 12, 25-27], we found no age-related decline in skill retention (in the main, secondary, as well as in the tertiary analysis, Figs. 2 and 6). The finding that old adults stabilize motor performance after 24 hours is in agreement with the few studies that reported retention data in old adults [10, 26]. Based on these studies, we expected the young adults to further improve their performance during the offline period but instead, they also stabilized their performance in the present study. Possibly, a ceiling effect prevented our young participants to further improve motor performance during the 24-hour offline period.

Cognitive processes, especially attention to procedural details of the task, contribute to motor learning [3, 28, 29]. Contrary to our hypothesis, we observed no age-related effects of sustained visual attention to a key element of the task, that is, observing the templates without physical movements. Motor performance in old_{AC} and $young_{AC}$ improved slightly from baseline to retention (4.2 and 3.6°, respectively), but this improvement did not differ between age groups. This improvement is probably mainly caused by familiarization of the task during the 3 tests because the no-intervention control group also increased performance slightly ($old_{control}$: 2.7°; $young_{control}$: 5.1°). The slightly higher increase in motor performance in old_{AC} versus $old_{control}$ may indicate that functional connectivity between brain areas associated with visuospatial attention (anterior-anterior areas and anterior-posterior areas of the cortex) is relatively intact in the sample of old adults participating in the present study, an indication in contrast with previous findings [22]. In summary,

actual motor execution is needed for skill acquisition and retention to occur, and such motor learning is probably affected minimally by the observational element as examined in the present study.

3.4.3 Age-related changes in neuronal mechanisms of motor skill acquisition

Because attentional control did not affect neuronal mechanisms after motor skill acquisition and at retention, the following sections focus on how age affects the neuronal mechanisms of the immediate and lasting effects of practicing a novel motor task. Furthermore, because the trends and direction of the changes in TMS data were similar in the main and tertiary analyses, we discuss the results from the main analysis only.

3.4.3.1 Changes in CSE during motor skill acquisition

Motor learning, including skill acquisition and retention, relies on use-dependent plasticity through long-term potentiation- (LTP-) like mechanisms. Use-dependent plasticity is influenced by N-methyl-D-aspartate (NMDA) receptor activation, γ-aminobutyric acid (GABA) inhibition, and cholinergic muscarinic receptor function [30, 31]. One way to index LTP-like mechanisms is by measuring CSE [30-32]. Motor practice can increase CSE, measured as the peak-to-peak amplitude of the MEP, in young [9, 11, 33, 34] and old adults [9]. CSE at rest did not change directly after motor practice in both age groups, similar to some [8 - right thumb] but not all studies [8 - left thumb, 9, 11]. There may be several reasons for a lack of change in CSE after motor practice in either age group. First, a magnetic pulse focuses on a small and selective brain area. Brain areas other than the targeted primary motor cortex (M1), such as premotor areas, cerebellum, posterior parietal cortex, visual cortex, and striatum, also become active during motor practice, but these areas cannot be probed by TMS [14, 35; for review see 19, 36]. Second, MEPs could be influenced by excitability changes of the spinal motoneuron pool caused by descending corticospinal pathways arriving from brain areas other than M1 [35]. Third, the use of different criteria for stimulation intensities (120% resting motor threshold [rMT] vs. MEP of 1 mV) and the expression of CSE (MEPs at 1 stimulation intensity vs. entire input-output curve) further complicate interpretation of CSE data. Finally, the small but significant baseline difference in CSE_{rest} between old_{MP} and $young_{MP}$ might have affected the changes in CSE_{rest} from baseline to posttest.

As a new approach, we measured CSE during the motor task. We expected that measurements of CSE in such "active" state versus at rest would be more specific and sensitive to changes induced by motor practice because M1 is active during task execution, which can be enhanced by inputs from premotor and supplementary secondary motor areas also activated by muscle contraction (for a review see [19]). Against this expectation, CSE during the task actually decreased by 22% in young adults, a finding consistent with a similar decrease reported by the only study that also measured CSE during (interleaved) practice of a motor task [37], and remained, as at rest, unchanged in old adults (Fig. 4B). The lack of CSE modulation, both at rest and during the task, in old adults may be explained by age-related physiological changes that are associated with motor learning. Data in animals and humans showed age-related seropositivity of NMDA receptor autoantibodies causing a decrease in the number of NMDA receptors [38], a loss of GABAergic interneurons (for a review see [39]), and a decrease of cholinergic muscarinic receptor binding [40]. Because NMDA, GABA, and muscarinic circuits are known to affect LTP-like mechanisms,

reductions in effectors of these mechanisms may have caused the lack of effects in CSE in old adults. In sum, even though previous studies assigned a putative role of M1 CSE in motor skill acquisition [9, 33, 41], the picture emerging from present and past studies is that CSE is probably a suboptimal TMS metric to probe the neuronal mechanisms of motor skill acquisition in old adults.

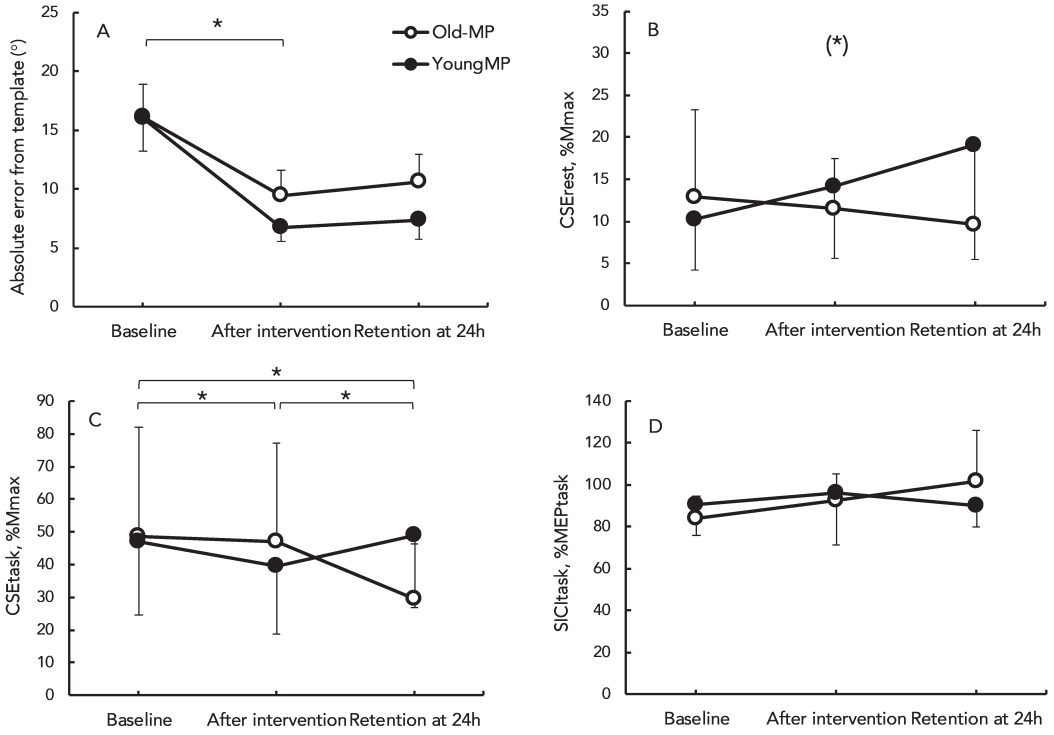

Fig. 6 Motor performance (A), CSE_{rest} (B), CSE_{task} (C), and short-interval intracortical inhibition during the task (D) of the 8 best old (open symbols) and 8 worst young adults (filled symbols) at baseline, after motor practice, and at retention. There was a significant age × time interaction for motor performance ($F_{2, 28}$ = 5.6, p = 0.009), and CSE during the task ($F_{2, 28}$ = 3.8, p = 0.033). There was a borderline significant age × time interaction for CSE measured at rest ($F_{2, 28}$ = 2.9, p = 0.072).*Change in $young_{MP}$ different from old_{MP}, (*) borderline age × time interaction. Abbreviations: CSE_{rest}, corticospinal excitability at rest; CSE_{task}, corticospinal excitability during task; M_{max}, maximal compound action potential; MEP_{task}, motor evoked potential during task; MP, motor practice group; $SICI_{task}$, short-interval intracortical inhibition during task.

3.4.3.2 Changes in inhibition during motor skill acquisition

Motor cortical inhibition tends to decrease with motor skill acquisition [9, 33, 37, 42], giving rise to the disinhibition hypothesis [37]. SICI is a GABA-A-mediated inhibition [43, 44]. Reduced GABA inhibition facilitates LTP-like mechanisms in the motor cortex [30, 45] and is associated with motor skill acquisition [46]. In agreement with some [8, 11] but in contrast to other motor learning studies [9], we observed no changes in SICI measured at rest in either age group. Although SICI per se did not change, relative motor skill acquisition correlated with an increase in inhibition measured at rest in old but not in young adults (Fig. 5B). However, there was no association when motor

skill acquisition was measured in absolute units. Overall, these results are not in line with the disinhibition hypothesis [37] after motor learning.

In contrast with SICI at rest, SICI measured during the task reduced in young adults, in line with the disinhibition hypothesis and results of a previous study [37]. Unlike in young adults, SICI during the task did not change after motor skill acquisition in old adults. The previously mentioned age-related loss of GABAergic interneurons may be a factor that accounts in part for a lack of modulation in SICI in old adults. Future studies will be needed to confirm the lack of effect of skill acquisition on SICI in aging and examine alternative measures of inhibition such as long-interval intracortical inhibition and short-latency afferent inhibition.

Although the correlations between relative motor skill acquisition and changes in SICI during the task in old and young adults only revealed trends, the opposite direction of associations in the 2 age groups (Fig. 5C) is noteworthy. In support of the disinhibition hypothesis, relative motor skill acquisition tended to correlate with a decrease in intracortical inhibition during the task in old adults. However, the association was in the opposite direction in young adults, although the group data showed a decrease in inhibition during the task. These results suggest that intracortical inhibition during the task is mediated differently in old and young adults during motor skill acquisition. However, these correlations should be taken with caution, as there were no associations when motor skill acquisition was measured in absolute units. So, the relationship between motor skill acquisition and changes in $SICI_{task}$ seems to depend much on whether motor skill acquisition is defined in absolute or relative units.

3.4.3.3 Changes in ICF during motor skill acquisition

Motor practice did not modify ICF measured at rest or during the task in either age group. These results agree with a lack of modification in ICF after motor practice in young adults [33, 34]. Although there were no changes in ICF, relative motor skill acquisition correlated with an increase in ICF during the task in old adults but with a decrease in young adults (Fig. 5E). Furthermore, when motor skill acquisition was expressed in absolute units, this relationship also was in opposite direction for old and young adults. These data suggest age-related changes in ICF modulation during motor skill acquisition. ICF is thought to reflect glutaminergic intracortical circuits [47], but there is some evidence that ICF can be modulated by GABAergic inhibition [43, 44, 48]. These studies and the correlation between changes in ICF_{task} and changes in $SICI_{task}$ (old: $r = 0.80$, $p = 0.003$; young: $r = 0.81$, $p = 0.001$; Supplementary materials) suggest that the correlation between changes in ICF_{task} and skill acquisition in young and old might be driven by changes in $SICI_{task}$.

3.4.4 Age-related changes in CSE and intracortical inhibition during motor skill retention

As in section 3.4.3, we confine the discussion to the neuronal changes in the motor practice groups and elaborate on the age-related changes that occurred in the offline period.

A main finding of the present study is that the direction of changes in CSE measured at rest and during the task and SICI measured during the task was opposite in old and young adults. In the offline period, CSE at rest decreased by 30% in old adults but increased by 23% in young adults. CSE measured during the task followed the same trend: it decreased by 27% in old adults but

increased by 52% in young adults. A greater increase in CSE in the offline period in comparison with the change from baseline to after motor practice is expected based on a previous study in young adults who performed interleaved practice [49]. The change in the opposite direction in old adults seems to agree with fMRI data showing age-related differences in brain connectivity patterns at a retention test 3 days after interleaved practice of a motor sequence, although behavioral improvements were similar in the 2 age groups [17]. In old adults, skill retention after interleaved practice correlated with higher functional connectivity between the right and left DLPFC and between the dorsal premotor cortex and inferior parietal lobule. In contrast, skill retention in young adults correlated with higher functional connectivity between DLPFC and the supplementary motor area and the inferior frontal gyrus. The present TMS and previous imaging data collectively suggest that different functional brain networks underlie skill retention in old versus young adults. Because both age groups showed similar magnitudes of skill retention, these results give rise to the compensation hypothesis [2].

SICI measured during the task decreased from 88% MEP_{task} after motor practice to 100% MEP_{task} at retention in old adults but increased from 100% MEP_{task} to 84% MEP_{task} in young adults (both $p < 0.05$). Because there was disinhibition after skill acquisition in young adults (section 3.4.3.2) but disinhibition after the offline period in old adults, it is suggested that disinhibition plays a role in motor learning in both age groups but that the temporal occurrence is age dependent. It is possible that GABA is modulated differently in old and young adults. GABA modulation in old adults may be associated with the consolidation of skill and not skill acquisition, as has been suggested in young adults [45]. However, further research is required to clarify this initial report concerning the offline neuronal changes probed for the first time with TMS in old and young adults.

3.4.5 Limitations

With retention measured at 24 hours only, it is unclear if the acquired motor memory traces could be activated days or weeks later and if age would affect this recall. Furthermore, the transfer of the acquired and retained skill to a task variant is not optimal, as the improvements in Purdue Pegboard performance were functionally minimal in both the age groups, suggesting that future studies should use perhaps a more relevant or specific transfer task than the Purdue Pegboard test. Another limitation was that we adjusted conditioning and test pulse intensity for determining SICI when the rMT or active motor threshold changed > 3 % stimulator output between tests. Other researchers have suggested measuring SICI at a constant TMS test intensity of an MEP of ~1mV, as test pulse intensity affects SICI [11]. However, we adjusted the test pulse intensity in a few cases to be able to deliver the test pulse at suprathreshold intensity. Finally, the results of this study should be taken with caution because we did not apply an alpha correction for multiple comparisons in the statistical analyses.

3.5 Conclusions

Old compared with young adults performed less well at baseline. Although the absolute magnitude of skill acquisition and retention was similar in the 2 age groups, skill acquisition but not retention was impaired in baseline-similar old compared with young adults. Furthermore, the

neuronal mechanisms differed between age groups, showing opposing effects when assessed by different measures. Motor skill acquisition was associated with increased ICF measured during the task in old adults but decreased ICF during the task in young adults. Furthermore, during skill retention, CSE measured at rest and during the task and inhibition during the task decreased in old but increased in young adults. In subsequent imaging studies, we expect to find that the age-related neurophysiological changes and differences in functional brain networks contribute to the age-related changes in corticospinal and intracortical excitability during motor learning.

References

1. World Health Organization. World Report on Ageing and Health. WHO Library Cataloguing-in-Publication Data; 2015. ISBN 978 92 4 069481 1.

2. Cabeza R, Anderson ND, Locantore JK, McIntosh AR. Aging gracefully: Compensatory brain activity in high-performing older adults. Neuroimage. 2002;17(3):1394-402.

3. Seidler RD, Bernard JA, Burutolu TB, Fling BW, Gordon MT, Gwin JT, et al. Motor control and aging: Links to age-related brain structural, functional, and biochemical effects. Neurosci Biobehav Rev. 2010;34(5):721-33.

4. Seidler RD. Neural correlates of motor learning, transfer of learning, and learning to learn. Exerc Sport Sci Rev. 2010;38(1):3-9.

5. Coats RO, Wilson AD, Snapp-Childs W, Fath AJ, Bingham GP. The 50s cliff: Perceptuo-motor learning rates across the lifespan. PLoS One. 2014;9(1):e85758.

6. Swinnen SP. Age-related deficits in motor learning and differences in feedback processing during the production of a bimanual coordination pattern. Cognitive Neuropsychology. 1998;15(5):439-66.

7. Zimerman M, Nitsch M, Giraux P, Gerloff C, Cohen LG, Hummel FC. Neuroenhancement of the aging brain: Restoring skill acquisition in old subjects. Ann Neurol. 2013;73(1):10-5.

8. Cirillo J, Rogasch NC, Semmler JG. Hemispheric differences in use-dependent corticomotor plasticity in young and old adults. Exp Brain Res. 2010;205(1):57-68.

9. Cirillo J, Todd G, Semmler JG. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. Eur J Neurosci. 2011;34(11):1847-56.

10. Brown RM, Robertson EM, Press DZ. Sequence skill acquisition and off-line learning in normal aging. PLoS One. 2009;4(8):e6683.

11. Rogasch NC, Dartnall TJ, Cirillo J, Nordstrom MA, Semmler JG. Corticomotor plasticity and learning of a ballistic thumb training task are diminished in older adults. J Appl Physiol (1985). 2009;107(6):1874-83.

12. Nemeth D, Janacsek K. The dynamics of implicit skill consolidation in young and elderly adults. J Gerontol B Psychol Sci Soc Sci. 2011;66(1):15-22.

13. Bennett IJ, Madden DJ, Vaidya CJ, Howard JH,Jr, Howard DV. White matter integrity correlates of implicit sequence learning in healthy aging. Neurobiol Aging. 2011;32(12):2317.e1.

14. Daselaar SM, Rombouts SA, Veltman DJ, Raaijmakers JG, Jonker C. Similar network activated by young and old adults during the acquisition of a motor sequence. Neurobiol Aging. 2003;24(7):1013-9.

15. Aizenstein HJ, Butters MA, Clark KA, Figurski JL, Andrew Stenger V, Nebes RD, et al. Prefrontal and striatal activation in elderly subjects during concurrent implicit and explicit sequence learning. Neurobiol Aging. 2006;27(5):741-51.

16. Schulz R, Zimerman M, Timmermann JE, Wessel MJ, Gerloff C, Hummel FC. White matter integrity of motor connections related to training gains in healthy aging. Neurobiol Aging. 2014;35(6):1404-11.

17. Lin CH, Chiang MC, Wu AD, Iacoboni M, Udompholkul P, Yazdanshenas O, et al. Enhanced motor learning in older adults is accompanied by increased bilateral frontal and fronto-parietal connectivity. Brain Connect. 2012;2(2):56-68.

18. Vallence AM, Goldsworthy MR. Can noninvasive brain stimulation enhance function in the ageing brain? J Neurophysiol. 2014;111(1):1-3.

19. Dayan E, Cohen LG. Neuroplasticity subserving motor skill learning. Neuron. 2011;72(3):443-54.

20. McNevin NH, Wulf G, Carlson C. Effects of attentional focus, self-control, and dyad training on motor learning: Implications for physical rehabilitation. Phys Ther. 2000;80(4):373-85.

21. Niendam TA, Laird AR, Ray KL, Dean YM, Glahn DC, Carter CS. Meta-analytic evidence for a superordinate cognitive control network subserving diverse executive functions. Cogn Affect Behav Neurosci. 2012;12(2):241-68.

22. Li Y, Li C, Wu Q, Xu Z, Kurata T, Ohno S, et al. Decreased resting-state connections within the visuospatial attention-related network in advanced aging. Neurosci Lett. 2015;597:13-8.

23. Berghuis KM, Veldman MP, Solnik S, Koch G, Zijdewind I, Hortobagyi T. Neuronal mechanisms of motor learning and motor memory consolidation in healthy old adults. Age. 2015;37:53.

24. Oldfield RC. The assessment and analysis of handedness: The edinburgh inventory. Neuropsychologia. 1971;9(1):97-113.

25. Roig M, Ritterband-Rosenbaum A, Lundbye-Jensen J, Nielsen JB. Aging increases the susceptibility to motor memory interference and reduces off-line gains in motor skill learning. Neurobiol Aging. 2014;35(8):1892-1900.

26. Korman M, Dagan Y, Karni A. Nap it or leave it in the elderly: A nap after practice relaxes age-related limitations in procedural memory consolidation. Neurosci Lett. 2015;606:173-6.

27. Nemeth D, Janacsek K, Londe Z, Ullman MT, Howard DV, Howard Jr JH. Sleep has no critical role in implicit motor sequence learning in young and old adults. Experimental brain research. 2010;201(2):351-8.

28. Raisbeck LD, Regal A, Diekfuss JA, Rhea CK, Ward P. Influence of practice schedules and attention on skill development and retention. Hum Mov Sci. 2015;43:100-6.

29. Wolpert DM, Diedrichsen J, Flanagan JR. Principles of sensorimotor learning. Nat Rev Neurosci. 2011;12(12):739-51.

30. Butefisch CM, Davis BC, Wise SP, Sawaki L, Kopylev L, Classen J, et al. Mechanisms of use-dependent plasticity in the human motor cortex. Proc Natl Acad Sci U S A. 2000;97(7):3661-5.

31. Sawaki L, Boroojerdi B, Kaelin-Lang A, Burstein AH, Butefisch CM, Kopylev L, et al. Cholinergic influences on use-dependent plasticity. J Neurophysiol. 2002;87(1):166-71.

32. Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, et al. Early consolidation in human primary motor cortex. Nature. 2002;415(6872):640-4.

33. Perez MA, Lungholt BK, Nyborg K, Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. Exp Brain Res. 2004;159(2):197-205.

34. Veldman MP, Zijdewind I, Solnik S, Maffiuletti NA, Berghuis KM, Javet M, et al. Direct and crossed effects of somatosensory electrical stimulation on motor learning and neuronal plasticity in humans. Eur J Appl Physiol. 2015;115(12):2505-19.

35. Bestmann S, Krakauer JW. The uses and interpretations of the motor-evoked potential for understanding behaviour. Exp Brain Res. 2015;233(3):679-89.

36. Lage GM, Ugrinowitsch H, Apolinario-Souza T, Vieira MM, Albuquerque MR, Benda RN. Repetition and variation in motor practice: A review of neural correlates. Neurosci Biobehav Rev. 2015;57:132-41.

37. Coxon JP, Peat NM, Byblow WD. Primary motor cortex disinhibition during motor skill learning. J Neurophysiol. 2014;112(1):156-64.

38. Busse S, Busse M, Brix B, Probst C, Genz A, Bogerts B, et al. Seroprevalence of N-methyl-D-aspartate glutamate receptor (NMDA-R) autoantibodies in aging subjects without neuropsychiatric disorders and in dementia patients. Eur Arch Psychiatry Clin Neurosci. 2014;264(6):545-50.

39. Lehmann K, Steinecke A, Bolz J. GABA through the ages: Regulation of cortical function and plasticity by inhibitory interneurons. Neural Plast. 2012;2012:892784.

40. Strong R, Hicks P, Hsu L, Bartus RT, Enna SJ. Age-related alterations in the rodent brain cholinergic system and behavior. Neurobiol Aging. 1980;1(1):59-63.

41. Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M. Role of the human motor cortex in rapid motor learning. Exp Brain Res. 2001;136(4):431-8.

42. Rosenkranz K, Kacar A, Rothwell JC. Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. J Neurosci. 2007;27(44):12058-66.

43. Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. Effects of antiepileptic drugs on motor cortex excitability in humans: A transcranial magnetic stimulation study. Ann Neurol. 1996;40(3):367-78.

44. Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W. The effect of lorazepam on the motor cortical excitability in man. Exp Brain Res. 1996;109(1):127-35.

45. Floyer-Lea A, Wylezinska M, Kincses T, Matthews PM. Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. J Neurophysiol. 2006;95(3):1639-44.

46. Stagg CJ, Bachtiar V, Johansen-Berg H. The role of GABA in human motor learning. Curr Biol. 2011;21(6):480-4.

47. Liepert J, Schwenkreis P, Tegenthoff M, Malin JP. The glutamate antagonist riluzole suppresses intracortical facilitation. J Neural Transm (Vienna). 1997;104(11-12):1207-14.

48. Ziemann U, Lonnecker S, Paulus W. Inhibition of human motor cortex by ethanol. A transcranial magnetic stimulation study. Brain. 1995;118(Pt 6):1437-46.

49. Lin CH, Knowlton BJ, Chiang MC, Iacoboni M, Udompholkul P, Wu AD. Brain-behavior correlates of optimizing learning through interleaved practice. Neuroimage. 2011;56(3):1758-72

Supplementary materials

Table S1. Correlations between baseline values of TMS variables and between percent changes in TMS variables in $Young_{MP}$

		MEPrest				MEPcontraction				SICIrest				SICItask				ICFtask			
		1	%Δ 1-2	%Δ 2-3	%Δ 1-3	1	%Δ 1-2	%Δ 2-3	%Δ 1-3	1	%Δ 1-2	%Δ 2-3	%Δ 1-3	1	%Δ 1-2	%Δ 2-3	%Δ 1-3	1	%Δ 1-2	%Δ 2-3	%Δ 1-3
MEPtask	1	0.51				0.06								0.14				-0.29			
	%Δ 1-2		0.45				0.13								-0.76*				-0.68*		
	%Δ 2-3			-0.05				0.15								-0.55				-0.43	
	%Δ 1-3				0.21				-0.45								-0.39				-0.49
MEPcontraction	1	0.13																			
	%Δ 1-2		0.04																		
	%Δ 2-3			-0.64*																	
	%Δ 1-3				-0.57																
SICIrest	1	0.19																			
	%Δ 1-2		-0.03																		
	%Δ 2-3			0.25																	
	%Δ 1-3				0.02																
ICFrest	1	-0.71*								0.05											
	%Δ 1-2		-0.22								0.18										
	%Δ 2-3			0.06								0.39									
	%Δ 1-3				-0.21								-0.16								
ICFtask	1													0.08							
	%Δ 1-2														0.81*						
	%Δ 2-3															0.89*					
	%Δ 1-3																0.65*				
CSP	1									-0.05				-0.04							
	%Δ 1-2										0.19				0.20						
	%Δ 2-3											0.09				0.00					
	%Δ 1-3												0.17				-0.09				

Values are Spearman's rho correlation coefficients. 1 = baseline, 2 = directly after intervention, 3 = retention test
* $p < 0.05$

Table S2. Correlations between baseline values of TMS variables and between percent changes in TMS variables in Old_{MP}

		MEPrest				MEPcontraction				SICIrest				SICItask				ICFtask			
		1	%Δ 1-2	%Δ 2-3	%Δ 1-3	1	%Δ 1-2	%Δ 2-3	%Δ 1-3	1	%Δ 1-2	%Δ 2-3	%Δ 1-3	1	%Δ 1-2	%Δ 2-3	%Δ 1-3	1	%Δ 1-2	%Δ 2-3	%Δ 1-3
MEPtask	1	0.24				-0.50								0.46				-0.33			
	%Δ 1-2		-0.33				-0.14								0.34				0.46		
	%Δ 2-3			-0.27				0.20								0.56				0.10	
	%Δ 1-3				0.02				-0.02								-0.11				-0.07
MEPcontraction	1	-0.39																			
	%Δ 1-2		-0.61*																		
	%Δ 2-3			-0.73*																	
	%Δ 1-3				-0.25																
SICIrest	1	-0.54																			
	%Δ 1-2		0.02																		
	%Δ 2-3			0.12																	
	%Δ 1-3				-0.22																
ICFrest	1	-0.48								0.64*											
	%Δ 1-2		-0.09								0.21										
	%Δ 2-3			-0.30								0.17									
	%Δ 1-3				-0.12								0.40								
ICFtask	1													-0.02							
	%Δ 1-2														0.80*						
	%Δ 2-3															0.42					
	%Δ 1-3																0.47				
CSP	1									0.29				0.11							
	%Δ 1-2										-0.26				-0.16						
	%Δ 2-3											-0.08				0.07					
	%Δ 1-3												0.38				0.11				

Values are Spearman's rho correlation coefficients. 1 = baseline, 2 = directly after intervention, 3 = retention test
* $p < 0.05$

I Chapter 4 I

Age-related changes in corticospinal excitability and intracortical inhibition after upper extremity motor learning: a systematic review and meta-analysis

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Abstract
It is unclear how old age affects the neuronal mechanisms of motor learning. We reviewed the neuronal mechanisms of how healthy old and young adults acquire motor skills as assessed with transcranial magnetic stimulation. Quantitative meta-analyses of 11 studies, involving ballistic and visuomotor tasks performed by upper extremity muscles in 132 healthy old and 128 young adults, revealed that the motor practice-induced increase in corticospinal excitability (CSE) is task-dependent but not age-dependent, with an increase in CSE in both age groups after visuomotor but not ballistic training. In addition, short-interval intracortical inhibition (SICI) is reduced in old but not young adults, but only after visuomotor practice. In addition, correlation analyses in 123 old and 128 young adults showed that the magnitude of motor skill acquisition did not correlate with increases in CSE or decreases in SICI in either age group. Thus, there are subtle age-related differences in use-dependent plasticity but increases in CSE or decreases in SICI are not related to motor skill acquisition in healthy young or old adults.

4.1 Introduction

Transcranial magnetic stimulation (TMS) is a noninvasive technique that can index neuronal mechanisms of motor learning. The amplitude of the motor-evoked potential (MEP) generated by single-pulse TMS quantifies corticospinal excitability (CSE) [1]. A change in CSE after motor practice is considered as an indicator of neuronal plasticity [2, 3]. Furthermore, when applied in a paired-pulse paradigm using short (1-5 ms) interstimulus intervals, TMS can indicate neuronal plasticity by probing the excitability of GABAergic inhibitory interneurons assessed with short-interval intracortical inhibition (SICI) [4, 5].

In young adults, there is an increase in CSE and a decrease in SICI following a short period of motor practice [3, 6-9]. How old age affects these indicators of motor learning-related plasticity is less clear. Because of unfavorable age-related modifications in the neuromuscular system, particularly in the processes involved in corticospinal plasticity, it is reasonable to expect that healthy old adults would exhibit reductions in motor learning-related plasticity [10-12]. However, the findings are inconsistent [13-15], suggesting that age has a complex effect on use-dependent plasticity. In addition, comparisons between existing studies are limited by small sample sizes (< 20 subjects per age group), and variations in methods in terms of the targeted muscle groups, motor tasks, and TMS parameters. Finally, there are inconsistencies between studies in young and old adults that show a correlation between the magnitude of motor learning and the changes in TMS parameters after motor practice [8] and studies that do not find such a correlation [3, 14]. Again, small sample sizes make it difficult to interpret these correlations.

Therefore, the purpose of this systematic review and meta-analysis was to determine how age affects TMS indicators of neuronal plasticity measured after motor learning in healthy adults. Considering the paucity of data concerning the effects of age on TMS parameters after motor skill retention, we focus on motor skill acquisition, the initial phase of motor learning. As a secondary aim, we also examined the association between changes in motor behavior and the accompanying changes in TMS parameters using a unique analysis that pooled individual data requested from the authors of 10 studies.

4.2 Methods

4.2.1 Literature search and selection criteria

We performed a systematic literature search in PubMed and Web of Science for the period from 1 January 1980 to 21 December 2016. In addition, we scanned reference lists of included papers for potentially relevant papers. The Supplementary materials show the detailed search syntax, using the main terms: motor learning, TMS, theta burst stimulation, and paired associative stimulation. Various TMS variables like corticospinal excitability, intracortical inhibition and facilitation, short-latency afferent inhibition, and cortical silent period were also included in the syntax. Inclusion criteria were as follows: English language, full text availability, publication within last 35 years, human subjects with a mean age >60 years, motor learning, behavioral outcomes, and TMS outcome measures. Studies in patients without a healthy control group, review articles, and meta-analyses were excluded. We screened titles, abstracts and, if necessary full texts to determine eligibility.

4.2.2 Coding of studies and quality assessment

Data were extracted for the intervention group that performed motor practice or the sham-control group when noninvasive brain stimulation such as transcranial direct current stimulation was used. Each study was coded for number of subjects, age, handedness, type and duration of intervention, the hand trained, target muscle for TMS, conditioning and test pulse intensity, interstimulus interval, motor performance, CSE (measured as peak-to-peak MEP amplitudes), and SICI. We assessed the methodological quality using the 'Quality assessment tool for before-after studies with no control group', a 12-question tool [16]. The overall methodological quality of each study was rated as "good", "fair" or "poor".

4.2.3 Request for individual data

We contacted authors of studies listed in Table 1 to provide individual subjects data for the same groups from which we extracted group mean data. Alternatively and for data unreported, we requested the exact group mean and standard deviations values to compute mean percent changes. We used the individual data to estimate the association between motor skill acquisition and changes in CSE and SICI across studies in young and old adults.

4.2.4 Data and statistical analysis

Based on the mean values reported in each study, or calculated based on individual data, we characterized the motor practice-induced effects of each study as percent change = (baseline – post)/baseline*(-100), with a positive change reflecting an increase in motor performance, CSE and SICI values (i.e., reduced inhibition). Percent changes reported in the results section are mean percent changes based on study mean computed changes. We quantified CSE from I/O curves as the MEP size measured at an intensity of 120% of resting motor threshold (RMT) [13, 14]. When SICI was evoked at multiple conditioning-pulse intensities [13, 14], we extracted SICI values corresponding to a conditioning intensity of 90% active motor threshold (AMT) because this intensity (%stimulator output; %SO) is thought to reflect the highest inhibition at rest that is not influenced by short-interval intracortical facilitation [17].

In individual meta-analyses, using random-effect models in Review Manager Version 5.3, we determined: (1) the effects of age on motor performance, CSE, and SICI at baseline and (2) the effects of motor practice on motor performance, CSE, and SICI in old and young adults separately, including tests for subgroup differences. The first meta-analyses compared old and young adults, and the second meta-analyses compared baseline and post-test data. Mean, standard deviation, and number of subjects were used as input to compute standardized mean differences (SMD), also known as Hedges' (adjusted) *g*. Because the search resulted in 2 categories of motor tasks (i.e., visuomotor and ballistic), all performed by upper extremity muscles, we performed meta-analyses separately for each task to maximize homogeneity. Weighting of the studies was applied in Review Manager. A positive SMD indicates greater motor skill acquisition, increase in CSE, or a decrease in inhibition. SMD values of $0.20 \leq 0.49$ indicate small, $0.50 \leq 0.79$ indicate medium, and ≥ 0.80 indicate large effects [18].

Table 1. Characteristics of the studies and motor skill acquisition

Reference	Group (n)	Age	Handedness/ hand trained	Task	Joint	Duration (min)	Motor performance			
							Baseline	After MP	Unit	%Δ
Berghuis et al., 2015 [19]	Old (11)	71.3	R / R	Visuomotor	Wrist	20	-17.9	-10.6	°	41
Berghuis et al., 2016 [20]	Young (12)	22.7	R / R	Visuomotor	Wrist	20	-14.5	-6.3	°	57
Buetefisch et al. 2015 [21]	Old (9)	65.2	R / R	Ballistic	Wrist	30	1.13	1.06[c]	g	-7
Cirillo et al., 2010 [13]	Young (12)	22	R / R	Ballistic	Thumb	10	9.8	35.6	m/s^2	262
	Old (14)	67					12.3	29.2		137
Cirillo et al., 2011 [3]	Young (16)	23	R / R	Visuomotor	Index finger	18	-2.9	-2.4	°	19
	Old (16)	67					-4.3	-3.5		18
Dickins et al., 2015[a] [22]	Young (20)	24.3	R[c] / D	Ballistic	Thumb	10	18.6	31.9	m/s^2	72
	Old (20)	70					16.0	21.2		33
Dickins et al., 2015[b] [22]	Young (20)	24.3	R[d] / D	Sequential	Fingers	10	18.4	22.2	#/30s	21
	Old (20)	70					14.6	17.0		16
Goodwill et al., 2015 [23]	Young (12)	22	22 R, 2 L / D	Visuomotor	Wrist	2.5	-21.8	-17.8	rmse	18
	Old (12)	66					-34.7	-28.6		18
Hinder et al., 2011 [15]	Young (18)	21.6	R / R	Ballistic	Index finger	10	22.4	39.9	m/s^2	78
	Old (12)	67.8					23.7	37.3		57
Hinder et al. 2013a[24]	Young (15)	21.2	R / L	Ballistic	Index finger	10	15.8	26.9	m/s^2	70
	Old (15)	70.3					11.2	13.8		23
Hinder et al., 2013b[25]	Young (9)	19.4	R / LR	Ballistic	Index fingers	10	11.5	23.1	m/s^2	100
	Old (9)	66.3					13.5	16.8		25
Rogasch et al., 2009 [14]	Young (14)	21.3	R / R	Ballistic	Thumb	10	12.2	33.9	m/s^2	177
	Old (14)	70.5					9.3	20.8		124

Abbreviations: D, Dominant; L, Left; MP, motor practice; R, Right; rmse, root mean square error.
[a,b] Same study, but different interventions within the study. Participants performed both interventions.
[c] Data estimated from figures in corresponding paper.
[d] One young subject was classified as ambidextrous.

Individual subject data were tested for normality using the Shapiro-Wilk test. Because the data were not normally distributed, associations between changes in neurophysiological and behavioral outcomes in individual subject data were estimated using Spearman's rho correlation coefficient (r_s). Additionally, r_s's were computed to examine associations between baseline motor performance, CSE, and SICI and changes in motor performance, CSE, and SICI (see Supplementary materials, Tables A1-3). All correlations were considered separately for young and old subjects but were considered both separately and combined for visuomotor and ballistic training tasks. Bootstrap confidence intervals (CIs) were calculated, setting the number of samples at 1000 and the CI level at 95%.

4.3 Results

4.3.1 Study characteristics

Fig. 1 summarizes the results of the systematic literature search in a flowchart. The search identified 1148 studies (PubMed: 1055; Web of Science: 93). Fifty-five duplicates were removed and 1080 articles were excluded due to eligibility. Three potentially relevant studies were identified in reference lists. After screening, 16 articles met the eligibility criteria. Five studies were excluded because the authors of these studies quantified motor skill acquisition based on involuntary responses to a TMS stimulus instead of voluntary movements [26-28], the intervention could not be categorized into the 2 types of motor learning for the meta-analyses [29], or the study did not include a group of older adults [30].

The 11 included studies examined 132 old and 128 young adults. Table 1 shows the characteristics of the studies and the magnitude of motor skill acquisition in the 2 age groups. Table 2 shows the TMS parameters. Subjects were healthy with a mean age of 68.1 ± 2.2 (range: 55 – 82, old) and 22.4 ± 1.9 years (range 18 – 35, young). Seven studies used ballistic motor tasks and 4 studies measured skill acquisition by visuomotor tasks. Ballistic motor tasks consisted of maximizing peak acceleration of the thumb [13, 14, 22], index finger(s) [15, 24, 25], or wrist [21] in response to an auditory tone that was set at 0.5 Hz. One study also included a rapid sequential finger-to-thumb opposition task [22]. In visuomotor tasks, subjects followed zig-zagged templates using wrist flexion and extension [19, 20] or index finger abduction and adduction [3]. In 1 study, participants had to track vertical movements of a target limb using wrist flexion and extension [23]. The practice sessions had a mean duration of 13 ± 7 minutes (range 2.5 – 30 min). Overall, the included studies had a "fair" methodological quality.

Because the results of old adults in 1 study [19] were compared with young adults in another study [20], we entered the data of these subjects in the meta-analysis as 1 study [20]. In another study, all subjects performed 2 interventions [22]. Therefore, this study was entered in the meta-analyses as if it were 2 studies. One of these 2 interventions was a sequential finger opposition task, which was included in the ballistic task category to include these results in the meta-analyses. In total, data of 152 old and 148 young subjects were entered in the meta-analyses.

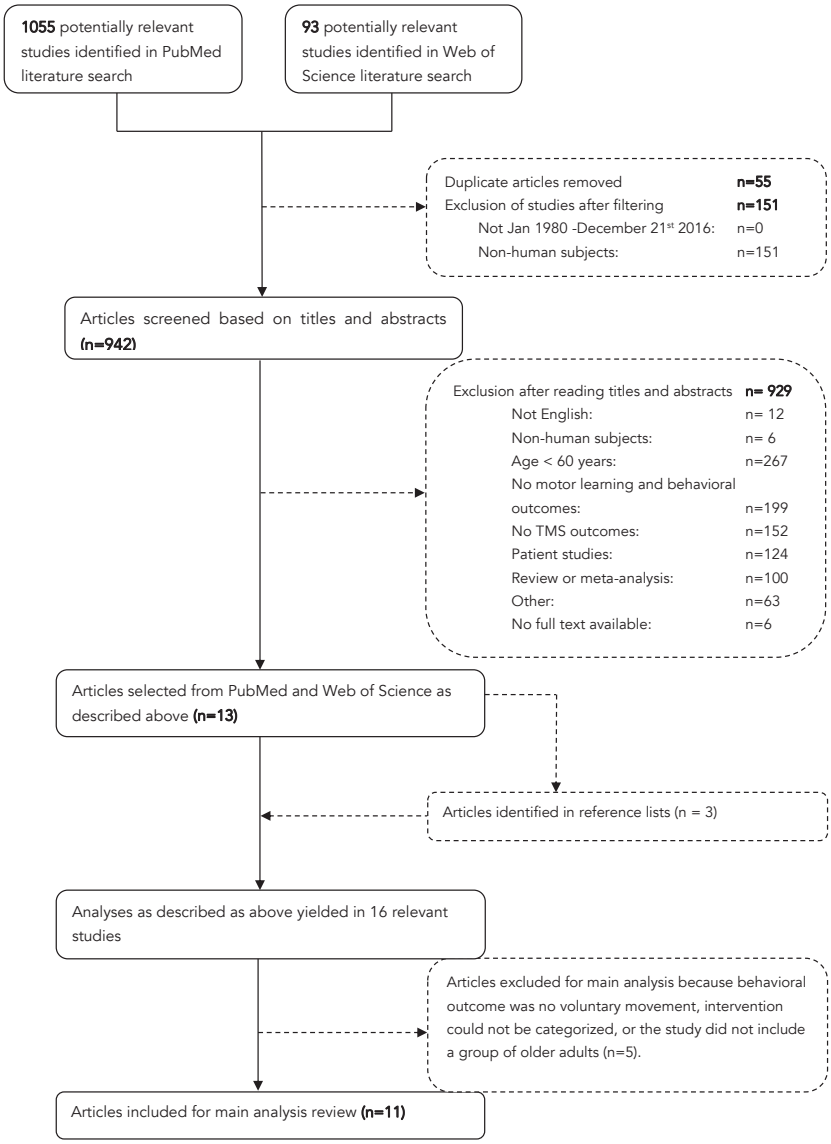

Fig. 1 Flowchart of systematic literature search in PubMed and Web of Science.

4.3.2 Effect of age on motor skill acquisition

At baseline, ballistic motor performance was not different between age groups ($p = 0.22$), whereas visuomotor performance was lower in old versus young adults ($p < 0.001$). Compared with baseline, both old and young adults performed better after each intervention (Fig. 2A and B, all p-values < 0.05), indicating that both age groups acquired the practiced skill. However, the magnitude of motor skill acquisition was lower in old compared with young adults after practicing ballistic motor tasks (old: 51%, young 111%; subgroup difference: $\chi^2 = 5.11$, $p = 0.02$) but unaffected by age after

practicing visuomotor tasks (old: 25%, young: 31%, p = 0.30).

4.3.3 Changes in corticospinal excitability after motor practice

TMS test-pulse intensity used to measure CSE was similar in old (57% ± 7%) and young (55% ± 5%) adults, ranging from 47 to 67 %SO (Table 2). At baseline, the MEP amplitude was similar in the 2 age groups in studies using ballistic (old: 0.82 mV; young: 0.99 mV; p = 0.20), and visuomotor tasks (old: 0.77 mV; young: 0.62 mV; p = 0.05). Fig. 3A shows that CSE did not change following ballistic training for either old (p = 0.18) or young (p = 0.31) adults, and that there was no between-age group difference (p = 0.83). Fig. 3B shows that after visuomotor practice, CSE increased in old (29%) and young (34%) adults (old: SMD: 0.55, 95% CI [0.10: 1.01], p = 0.02; young: SMD: 0.59, 95% CI [0.14: 1.04], p = 0.01), with no between-age group difference (p = 0.92).

4.3.4 Changes in short-interval intracortical inhibition after motor practice

Nine of the 11 studies measured SICI and used similar TMS settings in the 2 age groups (young: n = 108, old: n = 103). Test and conditioning-pulse intensity were, respectively, 60 ± 7%SO and 34 ± 5%SO in old and 58 ± 7%SO and 32 ± 4%SO in young adults, with an interstimulus interval of either 2 or 3 ms (Table 2). At baseline, the value of SICI was similar in old (51% ± 14% of TP) and young (50% ± 10% of TP) adults, independent of type of intervention (ballistic tasks: p = 0.65, visuomotor tasks: p = 0.22). Fig. 4A shows that SICI did not change in either age group after ballistic motor practice (old: p = 0.74; young: p = 0.60). After visuomotor practice, SICI values increased (i.e., a decrease in inhibition) by 20% in old adults (SMD: 0.59, 95% CI [0.06: 1.12], p = 0.03) but did not change in young adults (p = 0.34, Fig. 4B).

4.3.5 Correlation between motor skill acquisition and neuronal changes

Fig. 5 and Tables A1-3 (Supplementary materials) show the correlations between motor skill acquisition and changes in TMS variables based on individual data of 123 old and 128 young subjects. There was no association between improvements in motor performance and changes in CSE in both age groups (old: $r_{s\ 140}$ = -0.01, 95% CI [-0.20: 0.17]; young: $r_{s\ 146}$ = 0.05, 95% CI [-0.11: 0.20], both p-values > 0.05, Fig. 5A). There was also no correlation between motor skill acquisition and changes in SICI in old ($r_{s\ 85}$ = -0.16, 95% CI [-0.36: 0.05], p = 0.141) and young adults ($r_{s\ 91}$ = -0.20, 95% CI [-0.43: 0.03], p = 0.051; Fig. 5B). In separate correlation analyses for ballistic and visuomotor tasks, acquisition of a visuomotor skill correlated with a decrease in SICI value (i.e., more inhibition) in young ($r_{s\ 38}$ = -0.52, 95% CI [-0.72: -0.26], p = 0.001) but not old adults ($r_{s\ 37}$ = -0.17, 95% CI [-0.45: 0.17], p = 0.294). Acquisition of a visuomotor skill was unrelated to changes in CSE in both age groups (old: $r_{s\ 37}$ = -0.20, 95% CI [-0.54: 0.17]; young: $r_{s\ 38}$ = 0.05, 95% CI [-0.29: 0.35], both p-values > 0.05). Acquisition of a ballistic motor skill did not correlate with changes in either CSE or SICI in either age group (all p-values > 0.05).

4.4 Discussion

We reviewed the effects of age on the neuronal mechanisms of ballistic and visuomotor skill acquisition, as assessed with TMS. We found that: (1) motor practice-induced increases in CSE are task- but not age-dependent, with an increase in CSE after visuomotor but not after ballistic motor practice in both age groups; (2) motor-practice induced reductions in intracortical inhibition are

only task-dependent in old adults, showing reduced inhibition after visuomotor but not ballistic motor practice; and (3) the magnitude of motor skill acquisition is unrelated to increases in CSE and decreases in intracortical inhibition in either age group. We discuss these findings with a perspective on how age, the type of task performed, and the TMS parameters used to index plasticity each contributes to the mechanisms of motor skill acquisition.

4.4.1 CSE increases after visuomotor but not ballistic motor practice in both age groups

Against expectations, our quantitative analyses failed to identify a change in CSE (13%) after ballistic motor training in either age group (old: $p = 0.18$, young: $p = 0.31$). This result weakens the role played by CSE and M1 in the acquisition of a ballistic skill as shown in previous studies. Some studies showed that ballistic motor practice for less than 30 min can modulate CSE [14], that acquisition of such a skill is associated with an increase in CSE [31], and that repetitive TMS applied to M1 can disrupt ballistic skill acquisition [32]. In contrast, another study showed that ballistic skill acquisition is likely caused by spinal processes based on a transient increase in cervicomedullary MEPs to 248% of baseline immediately after practice, whereas visuomotor skill acquisition relies more on cortical processes, as reflected by unchanged cervicomedullary MEPs after practice [33]. In our CSE data (Table 2, Fig. 3), we cannot differentiate between motor cortical and spinal processes. We speculate that the specificity of CSE measurements could be increased by including measurements not only at rest but also during muscle contraction (see section 4.4.3).

In contrast to ballistic motor training, visuomotor training increased CSE similarly by 29% and 34% in old and young adults. We speculate that the more widespread patterns of activation during complex skill acquisition, like in pre-motor areas, supplementary motor areas, and cerebellum [34, 35], are associated with a greater involvement of brain areas upstream to M1, possibly resulting in a stronger modulation of CSE after visuomotor but not ballistic motor training. However, our outcome that there is no age-related difference in CSE modulation is unexpected as previous findings in animals [36] and humans [10, 37, 38] suggested that the ability of the brain to support plasticity-related phenomena declines with increasing age. Perhaps, the similar magnitude of visuomotor skill acquisition in old and young adults (respectively 25% and 31%, $p = 0.30$) explains the similar CSE modulation after visuomotor practice in the 2 age groups.

4.4.2 Reduction in intracortical inhibition only after visuomotor practice in old adults

Ballistic motor training did not affect SICI in either age group (old: 1.8%, young: 0.3%). A lack of change in SICI after ballistic skill acquisition may occur because selective activation of the target muscle is not required for this task, with selective muscle activation being a major contributor to SICI modulation [39]. Furthermore, perhaps TMS measures at rest are not specific or sensitive enough to detect age-related changes after ballistic motor skill acquisition (see section 4.4.3).

In contrast, there was a 20% decrease in inhibition when old adults practiced a visuomotor task (Fig. 4B). It is likely that SICI modulation plays a greater role in visuomotor compared with ballistic skill acquisition because visuomotor practice requires more precise and selective muscle activation to follow a template as accurately as possible [39]. However, it is surprising that cortical inhibition only decreased after visuomotor practice in old but not in young adults. This decrease in inhibition could suggest that cortical compensation occurred in old adults. Such compensation would be in

Table 2. TMS settings, values, and changes in young and old adults

Reference	Group (n)	Target muscle	CP intensity, %SO	TP intensity, %SO[c]	ISI, ms	MEP amplitude, mV			SICI value, %TP		
						Baseline	After MP	%Δ	Baseline	After MP	%Δ
Berghuis et al, 2015 [19]	Old (11)	ECR	43.9	65.9	2	0.35	0.39	11	44.8	53.7	20
Berghuis et al., 2016 [20]	Young (12)	ECR	38.5	57.8	2	0.28	0.35	25	66.0	53.9	-18
Buetefisch et al., 2015 [21]	Old (9)	ECU				0.65[d]	0.90[d]	39			
Cirillo et al., 2010 [13]	Young (12)	APB	33.6	54.1	3	0.82	1.08	31	46.2	38.7	-16
	Old (14)		33.8	53.9		1.03	0.96	-7	32.3	33.7	4
Cirillo et al., 2011 [3]	Young (16)	FDI	26.3	53.3	3	0.81	1.05	30	49.6	64.9	31
	Old (16)		28.1	61.8		0.95	1.25	32	49.9	53.3	7
Dickins et al. 2015[a] [22]	Young (20)	APB		53.7		0.99	1.06	7			
	Old (20)			50.1		0.97	1.20	24			
Dickins et al., 2015[b] [22]	Young (20)	APB		50.9		1.08	1.14	5			
	Old (20)			49.6		0.91	1.01	10			
Goodwill et al., 2015 [23]	Young (12)	ECR	29.4	47.8	3	0.78[e]	1.14[e]	47	51.6[e]	60.3[e]	17
	Old (12)		28.8	46.8		1.00[e]	1.44[e]	44	46.4[e]	61.5[e]	32
Hinder et al., 2011[15]	Young (18)	FDI	31.8	59.0	3	0.98	0.94	-4	42.0	49.0	17
	Old (12)		33.0	61.2		0.88	0.96	10	79	70	-11
Hinder et al., 2013a [24]	Young (15)	FDI	36.2	67.2		1.01	0.80	-21	63	71.2	13
	Old (15)		35.6	66.2		0.41	0.44	7	60	66	10
Hinder et al., 2013b [25]	Young (9)	FDI	30.8	57.2	3	1.43	1.37	-4	32	28	-13
	Old (9)		34.5	64.1		0.91	1.18	30	45	51	13
Rogasch et al., 2009 [14]	Young (14)	APB	32.4	52.8	3	0.60	1.04	74	46.7	46.6	0
	Old (14)		34.2	52.8		0.77	0.72	-7	46.8	43.4	-7

Abbreviations: APB, abductor pollicis brevis; CP, conditioning pulse; ECR, extensor carpi radialis; ECU, extensor carpi ulnaris; FDI, first dorsal interosseus; ISI, interstimulus interval; MP, motor practice; SO, stimulator output; TMS, transcranial magnetic stimulation; TP, test pulse.

[a,b] Same study, but different interventions within the study. Participants performed both interventions.

[c] When articles used different test-pulse intensities for determining corticospinal excitability and short-interval intracortical inhibition, the test-pulse intensity for determining corticospinal excitability is shown.

[d] Data estimated from figures in corresponding paper.

[e] Unlike the other the other articles, this article measured MEPs and SICI during a voluntary contraction of ±5% maximal root mean square error of the EMG.

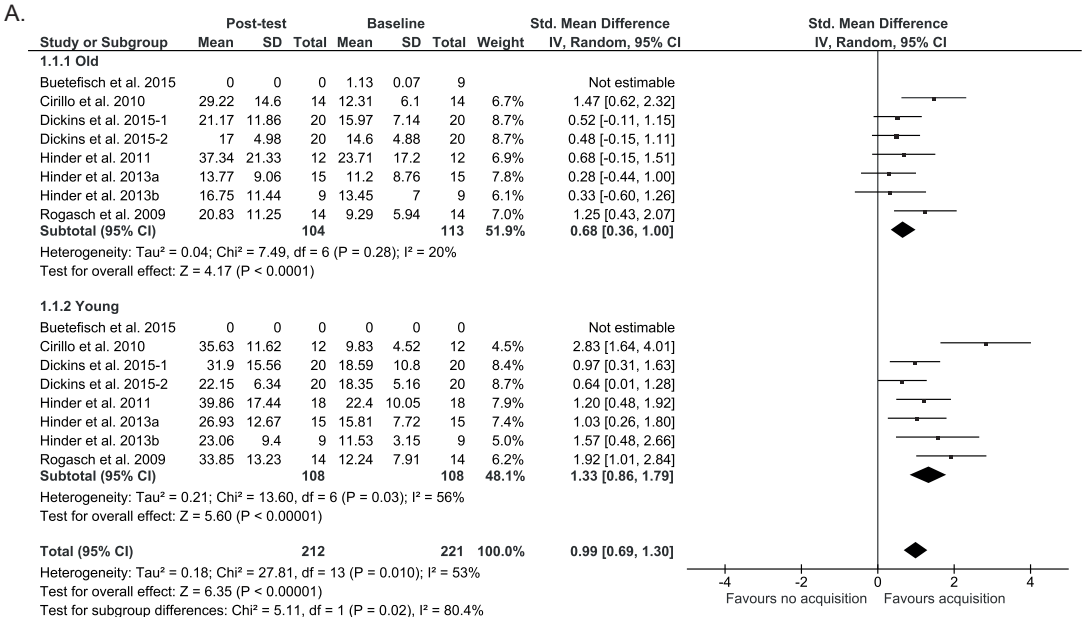

Fig. 2 Meta-analysis of the effect of motor practice of a ballistic motor task (A) and a visuomotor task (B) on motor performance in old and young adults. After ballistic motor practice, old and young adults improved their performance respectively by 51% and 111%. After visuomotor practice, old and young adults improved their performance respectively by 25% and 31%. Abbreviations: CI, confidence interval; IV, inverse variance; SD, Standard deviation.

line with a previous review showing that high- compared with low-performing old adults better modulate SICI [40]. In addition, there were no age-related differences in SICI at baseline in the 3 visuomotor studies, in agreement with some [41] but not all studies [42, 43]. This may have increased the capacity for SICI modulation in healthy old adults. However, young adults did not modulate SICI after visuomotor practice. The reason for this is currently unclear.

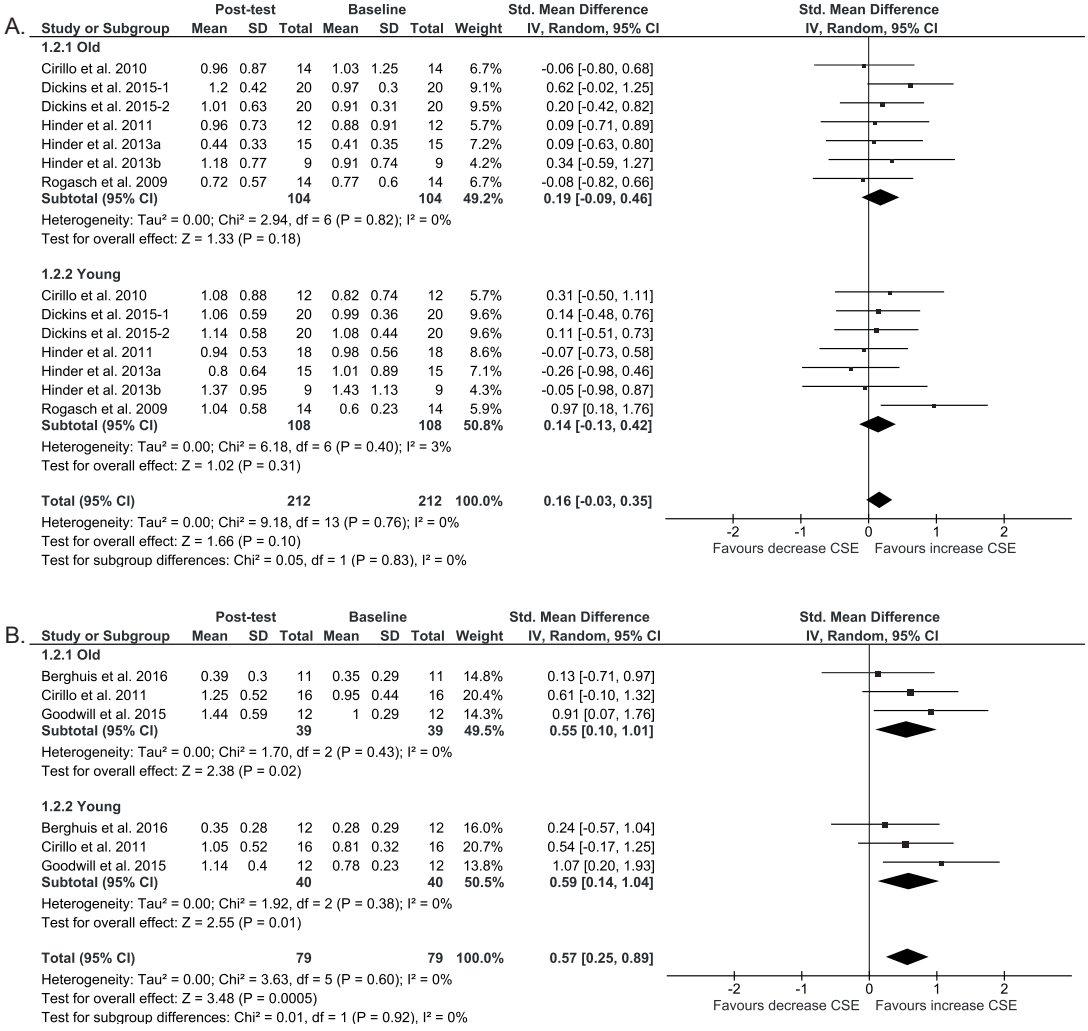

Fig. 3 Meta-analysis of the effect of motor practice of a ballistic motor task (A) and a visuomotor task (B) on corticospinal excitability in old and young adults. After ballistic motor practice, CSE did not change in both old (13%) and young adults (13%). After visuomotor practice, CSE increased in old (29%) and young adults (34%). Abbreviations: CI, confidence interval; CSE, corticospinal excitability; IV, inverse variance; SD, Standard deviation.

4.4.3 Motor skill acquisition tends to be unrelated to neuronal changes

There was no association between motor skill acquisition and increases in CSE or decreases in intracortical inhibition in either age group. Motor skill acquisition in young adults even tended to be associated with increases in intracortical inhibition (Fig. 5B). A recent review suggested several possible reasons for the lack of association between motor skill acquisition and modulations of CSE [44]. First, the relationship between these measures may be more complex than the linear association that is often expected. Second, the cortical elements targeted by TMS may not always be the same as the ones activated by volitional motor commands. Third, although changes in CSE

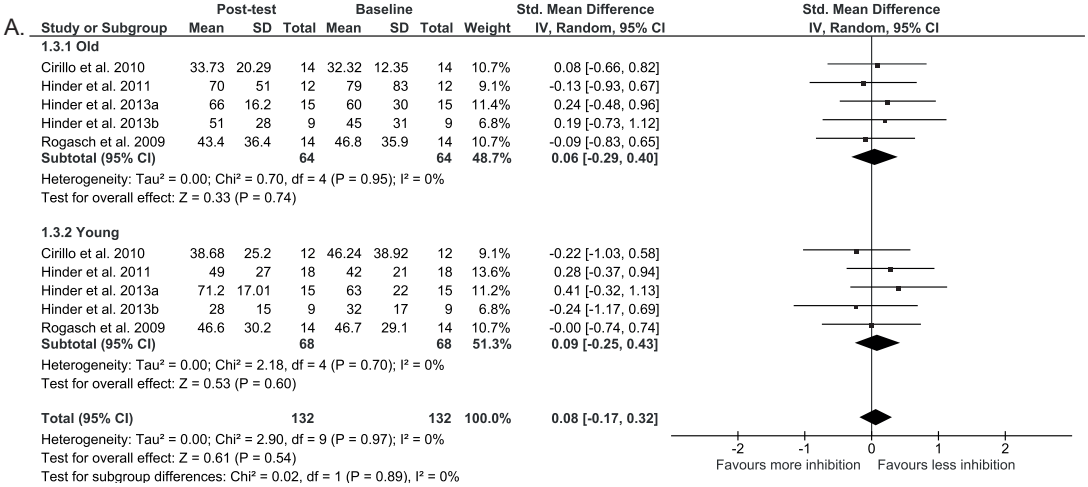

Fig. 4 Meta-analysis of the effect of motor practice of a ballistic motor task (A) and a visuomotor task (B) on short-interval intracortical inhibition in old and young adults. After ballistic motor practice, SICI did not change in both old (2%) and young adults (0%). After visuomotor practice, SICI values increased (i.e., less inhibition) in old (20%) but did not change in young adults (10%). Abbreviations: CI, confidence interval; IV, inverse variance; SD, Standard deviation; SICI, short-interval intracortical inhibition.

during motor skill acquisition can indicate modified neurophysiological processes, they may not provide causal information about motor behavior. Fourth, excitability changes in the spinal motoneuron pool caused by corticospinal pathways originating from brain areas other than M1 can influence MEP amplitudes. Finally, measures of CSE (e.g., MEP size) contain contributions from areas other than those activated when applying TMS to M1. Therefore, not only do MEPs indicate the excitability of areas probed by TMS (M1) but also include a read-out of upstream processes that are not necessarily related to movement execution, for example, decision-making processes [44] or motor learning [45]. For this reason, the over-activation in multiple brain areas generally seen with advancing age may weaken the already poor relationship between motor skill acquisition and modulations of CSE.

In addition, the result that both CSE and SICI modulations are not related to skill acquisition in either age group can perhaps be explained by the experimental approach. Even though measuring neuronal plasticity at rest is assumed to reflect neuronal mechanisms during motor skill acquisition [31, 46], it is not understood why motor practice would alter CSE and SICI in the resting state of the brain. Compared with at rest, measuring CSE and SICI during movement preparation, muscle contraction, or during the execution of the learned task itself would perhaps be more specific and sensitive [20, 47]. During movement preparation, CSE and SICI decreases in young adults [48], but this modulation is reduced with advancing age, which may contribute to the impaired motor function in old adults [47, 49]. Therefore, there might be an age-related association between motor skill acquisition and SICI modulation during movement preparation. Alternatively, measures during muscle contraction, or specific during execution of the learned task, would include converging (excitatory and inhibitory) inputs to M1 from areas sub-serving motor execution. In addition, as M1 is active during movement execution, it is reasonable to expect that CSE and SICI would change after motor practice only during the same active state of the brain while performing the practice. A recent attempt to measure CSE and SICI during the task execution in young and old adults, however, could not confirm that measures during the task were more specific and sensitive than at rest [20].

4.4.4 Limitations and recommendations

It is remarkable that only studies using upper extremity motor tasks were available to be included in the current review and meta-analysis. This indicates that there is a need for future studies examining age-related differences in TMS variables after motor learning using lower extremity muscles. Old adults in the included studies were healthy and relatively young (68.1 ± 2.2 years) but well above our age filter of >60 years. Thus, our results may not be directly translatable to older adults with or without pathological conditions. There was a moderate to high heterogeneity in skill acquisition between studies using visuomotor interventions (I^2: 51% –83 %, see Fig. 2B) [50, 51]. The 3 visuomotor interventions differed in duration (2.5–20 minutes) and in the joint used to perform the task (wrist or index finger). This could have some implications for the reliability of the summary results of visuomotor skill acquisition. In addition, ballistic and visuomotor studies used a variety of test-pulse intensity settings (MEP of ~1mV, 120% RMT, or 130% RMT), conditioning-pulse intensities (70% RMT, 80% RMT, 80% AMT, 90% AMT, or 50% inhibition), and interstimulus intervals (2 or 3 ms). Such methodological differences could perhaps have had some effects on changes in SICI in young adults after visuomotor practice (I^2 = 39%) but not in the other analyses ($I^2 \leq 25\%$). Another limitation was that subjects in one study practiced a bilateral ballistic task but the behavioral tests were performed in a unilateral task [25]. As the mechanisms involved in bilateral and unilateral training are not identical, and may be differentially affected by age [25], the findings of this study could have confounded the CSE and SICI values in the meta-analyses. However, this seems unlikely as the heterogeneity between studies using ballistic interventions was low ($I^2 \leq 3\%$) and the SMD for this study did not deviate from the other studies. Furthermore, it could be argued that the changes in CSE after visuomotor practice in young and old adults (4 studies, Fig. 3B), and the decreases in cortical inhibition in old adults (Fig. 4B) could be inflated by the large changes in CSE and SICI reported by one study involving sham-tDCS [23]. However, as there is some evidence that sham-tDCS does not increase CSE [52] or decrease inhibition [53],

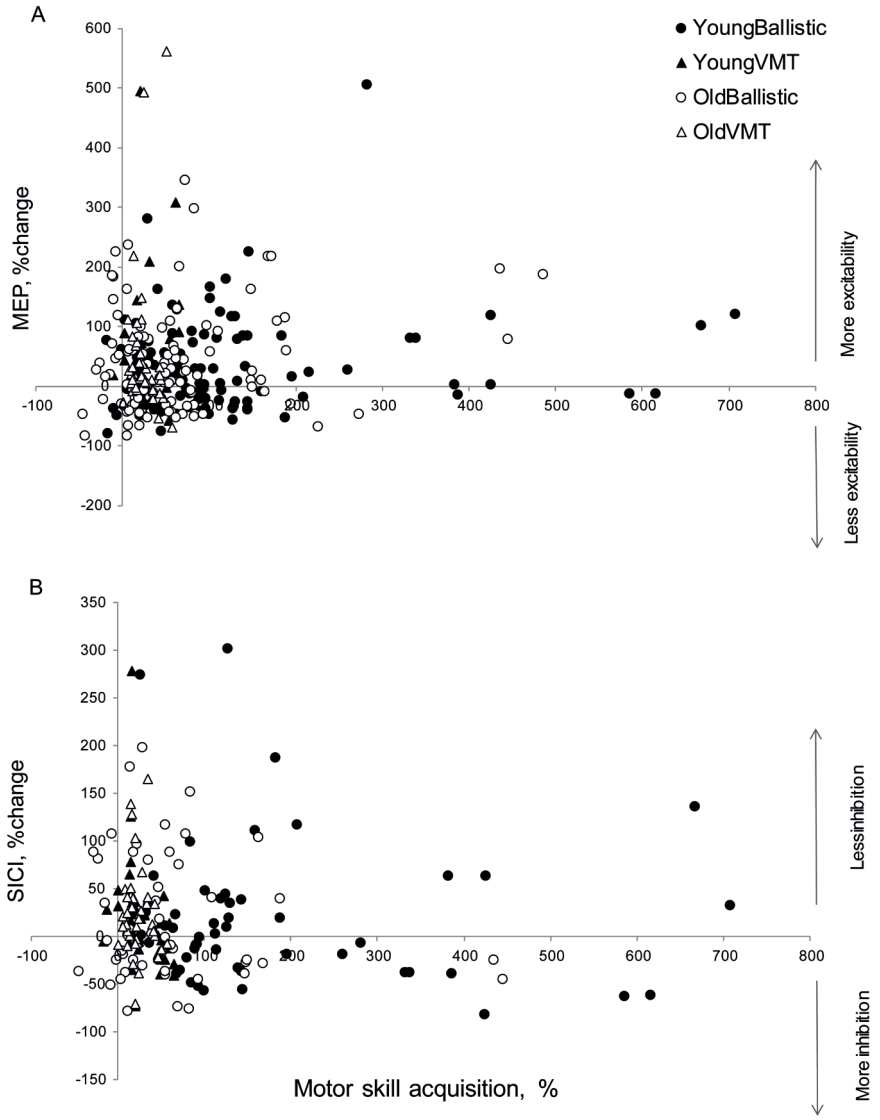

Fig. 5 Relationship between motor skill acquisition and changes in (A) corticospinal excitability, and (B) short-interval intracortical inhibition in old (open symbols, nA = 123, nB= 87) and young adults (filled symbols, nA = 128, nB = 93) performing ballistic (circles) and visuomotor tasks (triangles). Note: subjects from Dickins et al. (2015) [22] are shown twice in the graph because each subject performed 2 types of tasks.

it is unlikely that the placebo data would grossly bias the meta-analyses results. Another limitation is that the ballistic studies did not adjust the TMS intensities to match the MEP, RMT or AMT before and after training to compensate for the influences of MEP amplitude on SICI [54], whereas the visuomotor studies did. However, the within-study changes in CSE during ballistic motor skill acquisition probably did not have a major influence on the SICI results because, on average, CSE did not change in this group of studies (Fig. 3A). Finally, the low number of studies would normally require the use of a fixed instead of a random effects model [55]. However, we chose the random effects model as it was not plausible that all studies were conducted in the exact same way [55].

4.5 Conclusions

Increases in CSE are task- but not age-dependent. Visuomotor but not ballistic motor practice reduced SICI in old adults but SICI did not change in young adults in either task. Improvements in skill did not correlate with changes in CSE and SICI in either age group. Thus, there are subtle age-related differences in use-dependent plasticity but increases in CSE or decreases in SICI are not related to motor skill acquisition in healthy young or old adults. Compared with CSE and SICI measured at rest, TMS could be more effective to shed light on neuronal mechanisms of skill acquisition by measuring brain plasticity during the movement preparatory phase or during muscle contraction associated with the task.

References

1. Rothwell JC, Hallett M, Berardelli A, Eisen A, Rossini P, Paulus W. Magnetic stimulation: Motor evoked potentials. the international federation of clinical neurophysiology. Electroencephalogr Clin Neurophysiol Suppl. 1999;52:97-103.

2. Donoghue JP, Hess G., Sanes JN. Substrates and mechanisms for learning in motor cortex. In: Bloedel, J. Ebner T., Wise, SP, editor. Acquisition of motor behavior in vertebrates. MIT Press, Cambridge, MA, USA; 1996. p. 363-86.

3. Cirillo J, Todd G, Semmler JG. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. Eur J Neurosci. 2011;34(11):1847-56.

4. Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, et al. Corticocortical inhibition in human motor cortex. J Physiol. 1993;471:501-19.

5. Ziemann U, Muellbacher W, Hallett M, Cohen LG. Modulation of practice-dependent plasticity in human motor cortex. Brain. 2001;124(Pt 6):1171-81.

6. Perez MA, Lungholt BK, Nyborg K, Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. Exp Brain Res. 2004;159(2):197-205.

7. Veldman MP, Zijdewind I, Solnik S, Maffiuletti NA, Berghuis KM, Javet M, et al. Direct and crossed effects of somatosensory electrical stimulation on motor learning and neuronal plasticity in humans. Eur J Appl Physiol. 2015;115(12):2505-19.

8. Jensen JL, Marstrand PC, Nielsen JB. Motor skill training and strength training are associated with different plastic changes in the central nervous system. J Appl Physiol (1985). 2005;99(4):1558-68.

9. Leung M, Rantalainen T, Teo W, Kidgell D. Motor cortex excitability is not differentially modulated following skill and strength training. Neuroscience. 2015;305:99-108.

10. Busse S, Busse M, Brix B, Probst C, Genz A, Bogerts B, et al. Seroprevalence of N-methyl-D-aspartate glutamate receptor (NMDA-R) autoantibodies in aging subjects without neuropsychiatric disorders and in dementia patients. Eur Arch Psychiatry Clin Neurosci. 2014;264(6):545-50.

11. Lehmann K, Steinecke A, Bolz J. GABA through the ages: Regulation of cortical function and plasticity by inhibitory interneurons. Neural Plast. 2012;2012.

12. Strong R, Hicks P, Hsu L, Bartus RT, Enna SJ. Age-related alterations in the rodent brain cholinergic system and behavior. Neurobiol Aging. 1980;1(1):59-63.

13. Cirillo J, Rogasch NC, Semmler JG. Hemispheric differences in use-dependent corticomotor plasticity in young and old adults. Exp Brain Res. 2010;205(1):57-68.

14. Rogasch NC, Dartnall TJ, Cirillo J, Nordstrom MA, Semmler JG. Corticomotor plasticity and learning of a ballistic thumb training task are diminished in older adults. J Appl Physiol (1985). 2009;107(6):1874-83.

15. Hinder MR, Schmidt MW, Garry MI, Carroll TJ, Summers JJ. Absence of cross-limb transfer of performance gains following ballistic motor practice in older adults. J Appl Physiol (1985). 2011;110(1):166-75.

16. Quality assessment tool for before-after (pre-post) studies with no control group [Internet]; 2014. Available from: http://www.nhlbi.nih.gov/health-pro/guidelines/in-develop/cardiovascular-risk-reduction/tools/before-after.

17. Ortu E, Deriu F, Suppa A, Tolu E, Rothwell JC. Effects of volitional contraction on intracortical inhibition and facilitation in the human motor cortex. J Physiol. 2008;586(Pt 21):5147-59.

18. Cohen J. Statistical power for the behavioral sciences. Hillsdale: Lawrence Elbraum Associates; 1988.

19. Berghuis KM, Veldman MP, Solnik S, Koch G, Zijdewind I, Hortobagyi T. Neuronal mechanisms of motor learning and motor memory consolidation in healthy old adults. Age. 2015;37:53.

20. Berghuis K, De Rond V, Zijdewind I, Koch G, Veldman M, Hortobágyi T. Neuronal mechanisms of motor learning are age-dependent. Neurobiol Aging. 2016;46:149-59.

21. Buetefisch CM, Howard C, Korb C, Haut MW, Shuster L, Pergami P, et al. Conditions for enhancing the encoding of an elementary motor memory by rTMS. Clin Neurophysiol. 2015;126(3):581-93.

22. Dickins DS, Sale MV, Kamke MR. Intermanual transfer and bilateral cortical plasticity is maintained in older adults after skilled motor training with simple and complex tasks. Front Aging Neurosci. 2015;7:73.

23. Goodwill AM, Daly RM, Kidgell DJ. The effects of anodal-tDCS on cross-limb transfer in older adults. Clin Neurophysiol. 2015;126(11):2189-97.

24. Hinder MR, Carroll TJ, Summers JJ. Inter-limb transfer of ballistic motor skill following non-dominant limb training in young and older adults. Exp Brain Res. 2013;227(1):19-29.

25. Hinder MR, Carroll TJ, Summers JJ. Transfer of ballistic motor skill between bilateral and unilateral contexts in young and older adults: Neural adaptations and behavioral implications. J Neurophysiol. 2013;109(12):2963-71.

26. Sawaki L, Yaseen Z, Kopylev L, Cohen LG. Age-dependent changes in the ability to encode a novel elementary motor memory. Ann Neurol. 2003;53(4):521-4.

27. Celnik P, Stefan K, Hummel F, Duque J, Classen J, Cohen LG. Encoding a motor memory in the older adult by action observation. Neuroimage. 2006;29(2):677-84.

28. Massie CL, Kantak SS, Narayanan P, Wittenberg GF. Timing of motor cortical stimulation during planar robotic training differentially impacts neuroplasticity in older adults. Clin Neurophysiol. 2015;126(5):1024-32.

29. Lin CH, Chiang MC, Wu AD, Iacoboni M, Udompholkul P, Yazdanshenas O, et al. Age related differences in the neural substrates of motor sequence learning after interleaved and repetitive practice. Neuroimage. 2012;62(3):2007-20.

30. Zimerman M, Wessel MJ, Timmermann JE, Granstrom S, Gerloff C, Mautner VF, et al. Impairment of procedural learning and motor intracortical inhibition in neurofibromatosis type 1 patients. EBioMedicine. 2015;2(10):1430-7.

31. Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M. Role of the human motor cortex in rapid motor learning. Exp Brain Res. 2001;136(4):431-8.

32. Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, et al. Early consolidation in human primary motor cortex. Nature. 2002;415(6872):640-4.

33. Giesebrecht S, van Duinen H, Todd G, Gandevia SC, Taylor JL. Training in a ballistic task but not a visuomotor task increases responses to stimulation of human corticospinal axons. J Neurophysiol. 2012;107(9):2485-92.

34. Sadato N, Campbell G, Ibanez V, Deiber M, Hallett M. Complexity affects regional cerebral blood flow change during sequential finger movements. J Neurosci. 1996;16(8):2691-700.

35. Holmstrom L, de Manzano O, Vollmer B, Forsman L, Valero-Cuevas FJ, Ullen F, et al. Dissociation of brain areas associated with force production and stabilization during manipulation of unstable objects. Exp Brain Res. 2011;215(3-4):359-67.

36. Barnes CA. Long-term potentiation and the ageing brain. Philos Trans R Soc Lond B Biol Sci. 2003;358(1432):765-72.

37. Fathi D, Ueki Y, Mima T, Koganemaru S, Nagamine T, Tawfik A, et al. Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. Clin Neurophysiol. 2010;121(1):90-3.

38. Muller-Dahlhaus JF, Orekhov Y, Liu Y, Ziemann U. Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. Exp Brain Res. 2008;187(3):467-75.

39. Zoghi M, Pearce SL, Nordstrom MA. Differential modulation of intracortical inhibition in human motor cortex during selective activation of an intrinsic hand muscle. J Physiol. 2003;550(Pt 3):933-46.

40. Levin O, Fujiyama H, Boisgontier MP, Swinnen SP, Summers JJ. Aging and motor inhibition: A converging perspective provided by brain stimulation and imaging approaches. Neurosci Biobehav Rev. 2014;43:100-17.

41. Shibuya K, Park SB, Geevasinga N, Huynh W, Simon NG, Menon P, et al. Threshold tracking transcranial magnetic stimulation: Effects of age and gender on motor cortical function. Clinical Neurophysiology. 2016;127(6):2355-61.

42. Marneweck M, Loftus A, Hammond G. Short-interval intracortical inhibition and manual dexterity in healthy aging. Neurosci Res. 2011;70(4):408-14.

43. McGinley M, Hoffman RL, Russ DW, Thomas JS, Clark BC. Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. Exp Gerontol. 2010;45(9):671-8.

44. Bestmann S, Krakauer JW. The uses and interpretations of the motor-evoked potential for understanding behaviour. Exp Brain Res. 2015;233(3):679-89.

45. Li Voti P, Conte A, Suppa A, Iezzi E, Bologna M, Aniello MS, et al. Correlation between cortical plasticity, motor learning and BDNF genotype in healthy subjects. Exp Brain Res. 2011;212(1):91-9.

46. Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol. 1995;74(3):1037-45.

47. Heise KF, Zimerman M, Hoppe J, Gerloff C, Wegscheider K, Hummel FC. The aging motor system as a model for plastic changes of GABA-mediated intracortical inhibition and their behavioral relevance. J Neurosci. 2013;33(21):9039-49.

48. Bestmann S, Duque J. Transcranial magnetic stimulation: Decomposing the processes underlying action preparation. Neuroscientist. 2016;22(4):392-405.

49. Fujiyama H, Hinder MR, Schmidt MW, Tandonnet C, Garry MI, Summers JJ. Age-related differences in corticomotor excitability and inhibitory processes during a visuomotor RT task. J Cogn Neurosci. 2012;24(5):1253-63.

50. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002;21(11):1539-58.

51. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003;327(7414):557-60.

52. Ho KA, Taylor JL, Loo CK. Comparison of the effects of transcranial random noise stimulation and transcranial direct current stimulation on motor cortical excitability. J ECT. 2015;31(1):67-72.

53. Sasaki R, Miyaguchi S, Kotan S, Kojima S, Kirimoto H, Onishi H. Modulation of cortical inhibitory circuits after cathodal transcranial direct current stimulation over the primary motor cortex. Front Hum Neurosci. 2016;10:30.

54. Opie GM, Semmler JG. Age-related differences in short- and long-interval intracortical inhibition in a human hand muscle. Brain Stimul. 2014;7(5):665-72.

55. Borenstein M, Hedges LV, Higgins JP, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. Res Synth Methods. 2010;1(2):97-111.

Supplementary materials

The search syntax used to identify studies that examined the effects of age on manual motor learning and the ensuing changes immediately after motor practice in neuronal excitability measured by transcranial magnetic brain stimulation in healthy humans. The flowchart in Figure 1 shows that the search identified 11 studies that met inclusion criteria.

("motor skills"[Mesh] OR motor skill* [tw] OR motor learning [tw] OR motor skill learning [tw] OR motor skill acquisition [tw] OR motor performance [tw] OR motor behavior [tw] OR motor memory consolidation[tw] OR task learning[tw] OR sequence learning[tw] OR task-specific improvement* [tw] OR Visuo-motor task* [tw] OR Perceptuomotor task* [tw] OR Ballistic movement task* [tw] OR Finger tapping task* [tw] OR Coordination task* [tw] OR Serial Response Reaction Task* [tw] OR Sensorimotor task* [tw] OR practice-related task* [tw]) AND ("Transcranial Magnetic Stimulation"[Mesh] OR transcranial magnetic stimulation [tw] OR TMS [tw] OR rTMS [tw] OR repetitive TMS [tw] OR Paired Associative Stimulation [tw] OR PAS [tw] OR Theta Burst Stimulation [tw] OR TBS [tw] OR TMS measurements [tw] OR TMS parameters [tw] OR intracortical inhibition [tw] OR SICI [tw] OR LICI [tw] OR short-latency afferent inhibition [tw] OR SAI [tw] OR intracortical facilitation [tw] OR ICF [tw] OR cortical excitability [tw] OR motor evoked potentials [tw] OR MEP[tw] OR cortical silent period [tw]).

Table A. Correlations between baseline motor performance, baseline corticospinal excitability (MEP amplitude) and short-interval intracortical inhibition (SICI), changes in motor performance, and changes in MEP amplitude and SICI. Spearman's rho correlation coefficient (rs) are shown separately for old and young subjects and for visuomotor tasks (VMT) and ballistic tasks combined and separately A1-A3).

A1. VMT+ballistic combined

		Baseline motor performance		Baseline MEP amplitude (mV)		Baseline SICI (%TP)		%change in motor performance		%change in MEP amplitude (mV)		%change in SICI value (%TP)	
		Old	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Baseline motor performance	rs	1	1	-0.01	0.269**	-0.582**	-0.604**	0.013	0.110	0.037	-0.140	-0.146	-0.008
	p	.	.	0.903	0.001	0	0.000	0.88	0.183	0.662	0.089	0.178	0.937
	N	143	148	142	148	87	93	143	148	142	148	87	93
Baseline MEP amplitude (mV)	rs			1	1	-0.129	-0.326**	-0.101	-0.144	-0.408**	-0.479**	0.174	0.179
	p			.	.	0.233	0.001	0.233	0.081	0	0	0.106	0.087
	N			142	148	87	93	142	148	142	148	87	93
Baseline SICI (%TP)	rs					1	1	-0.185	-0.185	0.072	0.141	-0.224**	-0.163
	p					.	.	0.087	0.076	0.507	0.178	0.037	0.119
	N					87	93	87	93	87	93	87	93
%change in motor performance	rs							1	1	-0.009	0.048	-0.159	-0.203
	p							.	.	0.918	0.564	0.141	0.051
	N							143	148	142	148	87	93
%change in MEP amplitude (mV)	rs									1	1	-0.190	-0.112
	p									.	.	0.077	0.287
	N									142	148	87	93
%change in SICI value (%TP)	rs											1	1
	p											.	.
	N											87	93

* p < 0.05, ** p < 0.01

A2. VMT

		Baseline motor performance		Baseline MEP amplitude (mV)		Baseline SICI (%TP)		%change in motor performance		%change in MEP amplitude (mV)		%change in SICI value (%TP)	
		Old	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Baseline motor performance	rs	1	1	-0.064	0.134	0.231	-0.133	-0.073	-0.167	-0.110	-0.157	-0.362*	0.176
	p	.	.	0.699	0.409	0.156	0.413	0.661	0.303	0.504	0.334	0.023	0.277
	N	39	40	39	40	39	40	39	40	39	40	39	40
Baseline MEP amplitude (mV)	rs			1	1	-0.282	-0.388*	-0.468**	-0.674**	0.079	-0.294	0.162	0.408**
	p			.	.	0.081	0.013	0.003	0	0.632	0.065	0.324	0.009
	N			39	40	39	40	39	40	39	40	39	40
Baseline SICI (%TP)	rs					1	1	-0.052	0.293	-0.082	-0.102	-0.468**	-0.395*
	p					.	.	0.755	0.067	0.621	0.53	0.003	0.012
	N					39	40	39	40	39	40	39	40
%change in motor performance	rs							1	1	-0.203	0.048	-0.172	-0.519
	p							.	.	0.215	0.771	0.294	0.001
	N							39	40	39	40	39	40
%change in MEP amplitude (mV)	rs									1	1	0.068	-0.101
	p									.	.	0.681	0.535
	N									39	40	39	40
%change in SICI value (%TP)	rs											1	1
	p											.	.
	N											39	40

* $p < 0.05$, ** $p < 0.01$

A3. Ballistic

		Baseline motor performance		Baseline MEP amplitude (mV)		Baseline SICI (%TP)		%change in motor performance		%change in MEP amplitude (mV)		%change in SICI value (%TP)	
		Old	Young	Old	Young	Old	Young	Old	Young	Old	Young	Old	Young
Baseline motor performance	rs	1	1	0.028	0.137	-0.424**	-0.354**	-0.324**	-0.514**	0.142	-0.019	0.086	0.100
	p	.	.	0.775	0.158	0.003	0.009	0.001	0.000	0.152	0.842	0.560	0.476
	N	104	108	103	108	48	53	104	108	103	108	48	53
Baseline MEP amplitude (mV)	rs			1	1	-0.172	-0.300*	-0.055	-0.223*	-0.544**	-0.515**	0.089	0.076
	p			.	.	0.243	0.029	0.581	0.020	0.000	0.000	0.549	0.589
	N			103	108	48	53	103	108	103	108	48	53
Baseline SICI (%TP)	rs					1	1	0.490**	0.609**	0.000	0.289*	-0.324*	-0.160
	p					.	.	0.000	0.000	0.997	0.036	0.025	0.251
	N					48	53	48	53	48	53	48	53
%change in motor performance	rs							1	1	0.060	0.172	-0.082	-0.076
	p							.	.	0.547	0.074	0.578	0.590
	N							104	108	103	108	48	53
%change in MEP amplitude (mV)	rs									1	1	-0.277	-0.134
	p									.	.	0.057	0.339
	N									103	108	48	53
%change in SICI value (%TP)	rs											1	1
	p											.	.
	N											48	53

* $p < 0.05$, ** $p < 0.01$

I Chapter 5 I

Age-related changes in brain deactivation but not in activation after motor learning

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Abstract
It is poorly understood how healthy aging affects neural mechanisms underlying motor learning. We used blood-oxygen-level dependent (BOLD) contrasts to examine age-related changes in brain activation after acquisition and consolidation (24-hours) of a visuomotor tracking skill. Additionally, structural magnetic resonance imaging and diffusion tensor imaging were used to examine age-related structural changes in the brain. Older adults had reduced gray matter volume (628 ± 57 ml) and mean white matter anisotropy (0.18 ± 0.03) compared with young adults (741 ± 59 ml and 0.22 ± 0.02, respectively). Although motor performance was 53% lower in older (n = 15, mean age 63.1 years) compared with young adults (n = 15, mean age 25.5 years), motor practice improved motor performance similarly in both age groups. While executing the task, older adults showed in general greater brain activation compared with young adults. BOLD activation decreased in parietal and occipital areas after skill acquisition but activation increased in these areas after consolidation in both age groups, indicating more efficient visuospatial processing immediately after skill acquisition. Changes in deactivation in specific areas were age-dependent after consolidating the motor skill into motor memory. Young adults showed greater deactivations from post-test to retention in parietal, occipital and temporal cortices, whereas older adults showed smaller deactivation in the frontal cortex. Since learning rate was similar between age groups, age-related changes in activation patterns may be interpreted as a compensatory mechanism for age-related structural decline.

5.1 Introduction

Despite age-related neuroanatomical and neurophysiological changes, such as decline in gray and white matter volume [1], reduction in white matter integrity [2], and a loss of gamma-amino-butyric-acid interneurons [3], healthy older adults are still able to acquire and retain new motor skills. The magnitude of motor learning can even equal that of young adults when practicing a visuomotor tracking skill [4, 5]. Because detrimental age-related changes involve brain areas that are activated during motor learning [6, 7], it is reasonable to expect that older compared with young adults would rely on different neural mechanisms during motor learning. This might be suggestive of adaptive or compensatory strategies.

However, it is poorly understood how healthy aging affects the neural mechanisms underlying motor learning. Functional Magnetic Resonance Imaging (fMRI) studies showed that older and younger adults activate similar brain areas during sequential motor practice, such as sensorimotor, parietal, striatal and cerebellar areas but additionally, older adults activate frontal and temporal areas bilaterally [8, 9]. Because age does not seem to affect the rate of motor learning in these studies, the age-related changes in activation patterns may be interpreted as a compensatory mechanism for age-related structural declines, which include reductions in gray and white matter volume [1]. The additional bilateral brain activation seen in older adults also agrees with the hemispheric asymmetry reduction in older adults (HAROLD) model, which also has been suggested as a compensatory strategy [10, 11]. In contrast, when in the early stages of visuomotor adaptation participants learned to adapt to rotated visual feedback, older compared with younger adults acquired the skill less well and meanwhile showed reduced brain activation in sensory, frontal, temporal and occipital areas, in the cingulate gyrus, insular cortex and subcortical regions such as the caudate nucleus and thalamus [12]. These studies are in accordance with the Compensation-Related Utilization of Neural Circuits Hypothesis (CRUNCH) from the working memory literature. CRUNCH hypothesizes that the age-related decline in neural efficiency leads to compensatory recruitment of additional neural resources at low levels of cognitive demand [13]. Conversely, when cognitive demands increase, older adults would reach a ceiling-level of activity resulting in under-activation and under-performance compared with young adults.

Brain activation recorded during motor practice identifies the involvement of putative brain areas in motor learning. However, examining *changes* in brain activation over time provides more insights into the age-related differences in the adaptive mechanisms underlying motor learning. After implicitly acquiring a motor sequence, brain activation in temporal (including the hippocampus) and prefrontal areas has been shown to increase in older but decrease in young adults [14]. Furthermore, older compared with young adults showed greater increases of activation from the first to the second half of training in the dorsolateral prefrontal cortex bilaterally, and in the right superior frontal and left orbitofrontal cortex. In contrast, young adults showed greater increases of activation in the right striatum, thalamus, motor, and occipital cortex, and in the cerebellum, parietal, and insular cortex bilaterally [14]. Additionally, young compared with older adults showed greater decreases of activation in the right orbitofrontal area. After acquiring a motor skill, this skill needs to be consolidated into motor memory to be retained. One study reported that four hours after explicitly learning a motor sequence, brain activation increased in frontal, temporal

and parietal areas, hippocampus and cerebellum in older individuals, but it decreased in young adults when participants did not have the opportunity to take a nap [8]. When participants took a nap, results were in the opposite direction [8]. Taken together, these limited available data suggest that changes in brain activation after implicit and explicit motor sequence acquisition and consolidation are age-dependent, with increases in frontal and temporal brain areas of older adults but decreases in the same brain areas of young adults after both stages of motor learning. How brain activation changes after acquisition and consolidation of a visuomotor tracking skill differs between young and older adults is unknown.

To the best of our knowledge, no fMRI study to date has examined age-related changes in brain activation over time after both the acquisition and consolidation phase. Therefore, the current study examined the effects of age on brain activation changes after acquisition and consolidation (24-hours) of a visuomotor tracking skill. We hypothesized that the changes in brain activation would be age-dependent. Based on previous motor sequence studies that included visuomotor [14] and explicit learning [8] components similar to our visuomotor tracking task, we specifically expected increases in older but decreases in young adults in frontal and temporal activation after both the skill acquisition and consolidation phase. This age-dependent change in frontal and temporal activation might indicate an age-dependent reliance on cognitive control and memory while learning a motor skill. Furthermore, to be able to compare our current results with previous studies, we examined the effects of age on the average brain activation during visuomotor task execution. We expected older compared with young adults to show greater activation when executing the visuomotor task. We tested both hypotheses with a whole-brain analysis approach. When motor learning rates are similar between young and old adults, as expected based on previous findings using a similar task [4], any age-related differences in brain activation or in task-related modulation of brain activity would be interpreted as an alternative, compensatory strategy in older adults.

5.2 Methods

5.2.1 Participants

Healthy young (n=17, 7 males, age range: 21 – 31, mean ± SD: 25.5 ± 2.3 years) and older (n=16, 9 males, age range: 56 – 72; 62.6 ± 5.3 years) right-handed [15] adults participated in this study. None of them had any contraindications to undergo MRI scanning or suffered from any pain or movement restriction in their right arm. Older adults were physically and cognitively preserved, according to the Groningen Activity Restriction scale (mean score: 18.9 ± 2.5, [16]) and the Mini Mental State Examination (mean score: 29.2 ± 0.8, 28 – 30, [17]). The study was approved by the IRCCS Santa Lucia Foundation Ethics Committee in Rome, Italy, and each participant signed an informed consent in accordance with the Declaration of Helsinki prior to enrollment.

5.2.2 Procedure

Fig. 1 shows the study design, which included two sessions, with approximately a 24-hour period in between. Participants underwent fMRI during visuomotor tracking task performance to determine brain activation before motor practice, after motor skill acquisition and after motor memory consolidation. Day 1 started with three familiarization trials of the visuomotor task. Subsequently,

participants executed a pre-test, a training session and a post-test inside the MRI scanner. Each test consisted of six blocks. Participants started each block by viewing a fixation cross for 20 s, followed by performing five trials of the experimental condition, viewing of the fixation cross for 20 s, and concluding the block by performing five trials of the control condition (see section 5.2.3). Both pre- and post-test consisted of the same trials that appeared in a pseudorandomized order. During both the pre- and post-test, fMRI acquisition was performed. The training session consisted of four blocks of 30 trials with 30 s of fixation cross between the blocks and took place inside the MRI scanner without fMRI acquisition. At the end of the session on Day 1, an anatomical scan was acquired as an anatomical reference (see section 5.2.4). As sleep is known to have an influence on motor learning, which could be different across age groups (e.g. [8]), we investigated whether sleep quality was similar in the two age groups. Therefore, on Day 2 (approximately 24 hours after Day 1), all participants filled in the Pittsburgh Sleep Quality Index (PSQI) questionnaire concerning sleep quality and quantity over the last month and last night. Subsequently, they entered the scanner for the final fMRI session during the retention test, which consisted again of the same trials as the pre- and post-test but in a different order. Finally, Diffusion Tensor Imaging (DTI) was conducted, so that we could determine whether our group of older adults showed expected age-related changes in white matter microstructural integrity [18].

5.2.3 Visuomotor task

Participants performed a visuomotor tracking task using an MR compatible manipulandum [19]. The manipulandum was affixed to the right side of the MR table, and the distance was adjusted to the participant's arm length. The settings were adjusted so that participants were only able to perform wrist flexion and extension in the transverse plane. Participants' right forearm was placed on cushions and participants held the grip of the manipulandum with the thumb taped to the fingers, reminding participants to perform the task with wrist- and not finger-movements. Head movements were minimized by using an adjustable padded head holder and foam pads.

The visuomotor task consisted of tracking templates using wrist flexion and extension [20]. There were two conditions: the experimental condition consisted of zigzagged templates with four or five turns, whereas the control condition consisted of monotonically increasing or decreasing templates. The templates were presented in white on a dark blue background, and the participants' wrist position was shown in green. The experimental and control condition each had five patterns and a duration of 4, 5 or 6 s. There was a 500 ms delay between trials. The pattern and duration of the templates were pseudorandomized, such that the mean duration of a five-trial block was 5 s and that all five patterns of either the experimental or the control condition appeared once within each five-trial block. The training session consisted of five different patterns but had a similar level of difficulty as those used for the experimental condition. These trials had similar durations as the testing trials and again the patterns and durations varied pseudorandomly. The visuomotor task was projected on a screen at the head end of the MRI scanner and visible for participants through a mirror affixed to the head coil. The start of the visuomotor test-trials was synchronized with the MRI scanner by waiting for a specific slice number that was communicated from the scanner to the laptop managing the visuomotor task software.

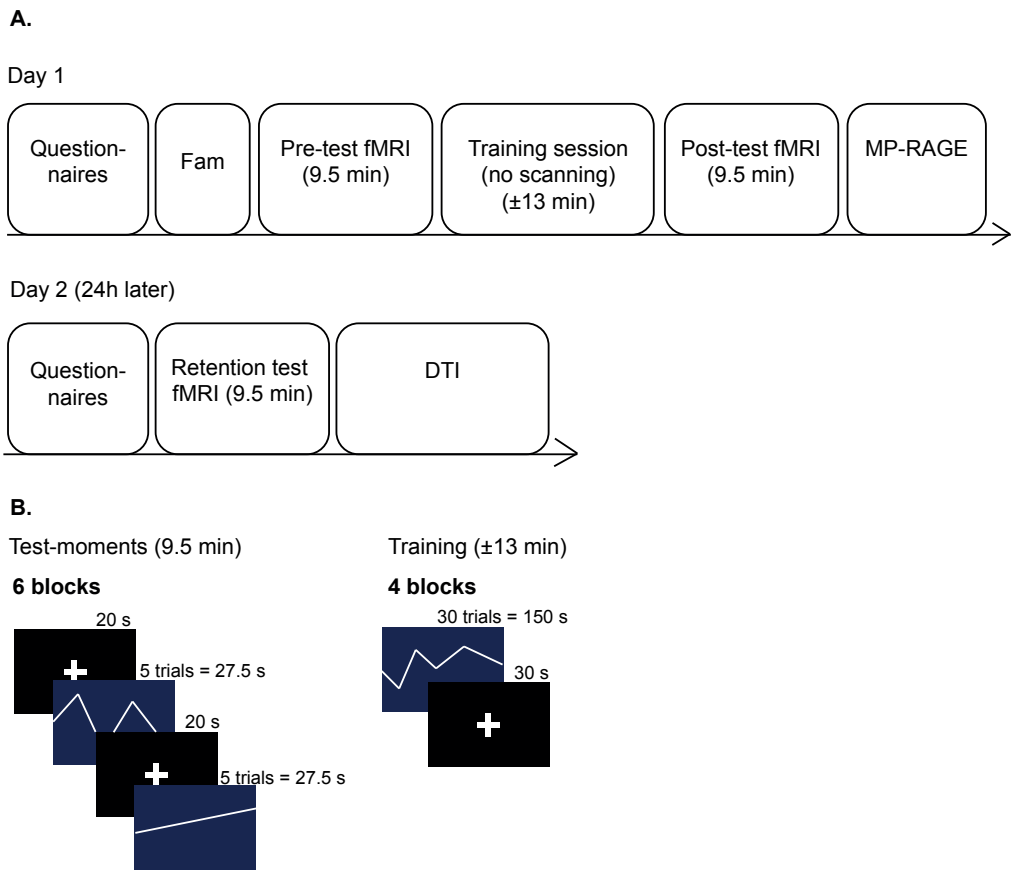

Fig. 1 The design of the study, with A) the complete study design, and B) the design for the visuomotor task in de MRI scanner with a zig-zagged experimental condition and a monotonically in- or decreasing line as a control condition. Fam, familiarization; fMRI, functional magnetic resonance imaging; MP-RAGE, Magnetization-Prepared Rapid Gradient-Echo sequence; DTI, diffusion tensor imaging.

5.2.4 fMRI and DTI

Brain imaging was performed with a Siemens Magnetom Allegra 3-T head-only scanning system (Siemens Medical Systems, Erlangen, Germany), equipped with a quadrature volume RF head coil. Blood-oxygen-level dependent (BOLD) contrasts were obtained using echo-planar T2*-weighted imaging with 32 slices (EPI; TR = 2.08 s, TE = 30 ms, flip angle = 70°, matrix 64 × 64, voxel size = 3 × 3 mm in-plane, slice thickness = 2.5 mm; 50% distance factor; FOV = 192 mm) providing coverage of the whole cerebral cortex. Per test, 279 functional volumes were acquired. In addition, we acquired a Magnetization-Prepared Rapid Gradient-Echo (MPRAGE) sequence as an anatomical reference (TR = 2.5 s, TE = 2.74 ms, voxel size 1 × 1 × 1 mm, matrix resolution 256 × 256 × 176, axial acquisition). Finally, DTI images were acquired using the following parameters: TR = 7000 ms, TE = 85 ms, 61 diffusion directions, maximum b factor = 1000 s/mm^2, isotropic resolution 2.3 mm^3. Sixty-one diffusion weighted images and seven non-diffusion weighted image (b = 0 s/mm^2) were acquired.

5.2.5 Data and statistical analyses

5.2.5.1 Analysis of participants' characteristics
Participants' characteristics were analyzed using SPSS version 24 (IBM SPSS Statistics, Armonk, NY, USA). Group differences between categorical variables (e.g. sex) were assessed with the $\chi 2$ test. Comparisons between older and young participants for continuous variables were performed using two-tailed independent t-tests. When continuous variables were not normally distributed, or in case of ordinal variables, Mann-Whitney's *U* test was performed. Significance was accepted at $\alpha = 0.05$.

5.2.5.2 Analysis of visuomotor task performance
The performance on the visuomotor task was analyzed in Matlab 2011a (The Mathworks Inc., Natick, MA, USA) for the experimental and control condition separately by calculating the absolute mean error of the participant's wrist joint position from the preprogrammed template. The performance value indicates an average error over the 30 trials per condition per test. A second order low-pass Butterworth filter of 5 Hz was used to filter the joint position data. Data obtained during the first second of each trial were discarded as it contained errors associated with reacting to the appearance of the template. The visuomotor performance data were not normally distributed and were therefore log-transformed. Mixed analysis of variance (ANOVA) was performed in SPSS with between-subjects factor age (young, older) and within-subjects factors time (pre, post, retention) and condition (experimental, control). Significance was accepted at $\alpha = 0.05$. The non-transformed data are reported in the results.

5.2.5.3 fMRI preprocessing and first level analysis
(f)MRI data preprocessing and first-level analysis were performed in SPM12 (Wellcome Trust Center for Neuroimaging, UCL, UK). The first four images of each EPI sequence were discarded to ensure T1 signal equilibrium. First, all functional images of each MRI session (Day1: pre + post and Day2: retention) were manually reoriented. Subsequently, all functional images from all sessions were realigned to the first image of the first session and co-registered to the mean functional image. Then, images were normalized to the MNI template and a 3D Gaussian kernel of 8mm full-width at half maximum (FWHM) was used to smooth the EPI images. Furthermore, the structural image of each participant was segmented to extract gray matter, white matter, and cerebrospinal fluid volumes. Age-related differences in these volumes were examined using an independent t-test in SPSS.

The exact onsets and durations of the experimental and control blocks of each participant were determined in Matlab using the experimental log-file. In the first-level analysis, brain activation during task execution (Experimental, Control) was modelled by a general linear model (GLM) for each test-moment (pre, post, retention) and participant. Six motion regressors were included in the design matrix for each test-moment to control for any head movements of the participant during the scanning sessions. High-pass filtering was implemented in the design matrix using a cutoff period of 128 s to remove low-frequency drifts from the time series. Statistical parametric maps (SPMs) were computed on subject level (F and t-statistics). Contrasts that were defined in the first level analysis and used in the second level analysis were as follows: activation per condition

versus baseline (e.g. ExperimentalPre), and activation at each time point with higher activation in experimental versus control condition (e.g. $Pre_{Experimental>Control}$). After the first-level analysis, Artifact Detection Tool (ART) was applied in all participants to check and correct for movement artifacts (https://www.nitrc.org/projects/artifact_detect). As the participants were explicitly told to move inside the scanner by acting on the manipulandum and considering that head motion-induced artifacts increase with age [21], we adopted a liberal threshold to identify outliers for the global signal intensity and head motion (z-threshold = 9; movement threshold =2 mm) while retaining an acceptable amount of data. None of the subjects had >10% outliers. No correlations between motion and timing of the experimental and control condition were detected. Subsequently, the first-level analysis was performed again, including the outliers and regressors computed by ART in the model as covariates of no interest.

5.2.5.4 fMRI second level analysis

Second level analyses were performed using linear mixed effects analyses (3dLME, implemented in AFNI, http://afni.nimh.nih.gov/afni/, [22]) because of missing data in one participant. We defined four linear mixed effects models. In the first model, we were interested in 1) the differences in brain activation between young and older adults during execution of both the experimental (Exp) and control (Contr) conditions to test the hypothesis that older adults utilize more brain activation compared with young adults in order to perform the visuomotor task and 2) the differences in brain activation between the conditions during task execution in order to examine whether there was greater brain activation when executing the experimental versus the control condition, which serves as input data for the second model. Therefore, functional images of each condition and time (ExpPre, ExpPost, ExpRetention, ContrPre, ContrPost, ContrRetention) after movement artifact correction were implemented per participant as input images in this model. Age, condition, and time were fixed factors. The intercept was allowed to vary across participants and was therefore a random factor. The covariance structure was an identity matrix. The following contrasts were computed using two-sided t-tests: Older > young and experimental > control.

In the second model, we were interested in changes in brain activation over time, specific for the experimental condition. Therefore, we entered the Experimental > Control contrasted images for each participant and each time-point in an ANOVA ($Pre_{Exp>Contr}$, $Post_{Exp>Contr}$, $Retention_{Exp>Contr}$) to examine the main effect of time (pre, post, retention) and the age × time interaction (hence, corrected for control condition activation). Age and time were fixed factors, and similar to model 1, the intercept was allowed to vary randomly across participants and the covariance structure was an identity matrix. The following post-hoc t-tests (two-sided) were specified in the model using activation masks representing the main and interaction effects to further examine the meaning of these effects: 1) changes in brain activation from one time point to another, averaged across age groups (post > pre, retention > post, and retention > pre); 2) differences between young and older adults in changes in brain activation over time, for example $Young_{Retention>Post}$ > $Older_{Retention>Post}$; and 3) differences between time points within age groups, for example $Young_{Retention}$>$Young_{Post}$.

In addition to these first two models, two models were defined that were replicas of the first two models but now inserted whole-brain gray matter volume (%total intracranial volume) as a covariate to examine whether the expected age-related differences in gray matter had an influence

on brain activation.

In all models, Monte Carlo simulation was used to correct for multiple comparisons and to determine the significant effects at cluster-level (3dClustSim, implemented in AFNI, initial threshold of $p = 0.001$, cluster size $k > 30$, 10000 iterations). Post-hoc contrasts in the second model were calculated with an uncorrected p-threshold of 0.001. Of these contrasts, only clusters greater than 10 voxels are reported in the text. Significant clusters were labelled using the Automated Anatomical Labeling atlas in MRIcron.

To understand the main and interaction effects of the fMRI analyses better, we extracted the parameter estimates of the GLM for each participant. For each cluster of the main and interaction effects, a mask was created. For each mask, mean parameter estimates were extracted in Matlab for each condition and time point in each participant.

5.2.5.5 Analysis of diffusion weighted images

Diffusion weighted images were preprocessed using tools from the FMRIB Software Library (FSL, University of Oxford, UK; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) and Camino (Microstructure Imaging Group, UCL, UK; http://camino.cs.ucl.ac.uk/). Diffusion weighted data were corrected for eddy current distortions and involuntary movements by affine coregistration using the FLIRT tool (part of the FMRIB Software Library). The b matrices were rotated accordingly [23]. The diffusion tensor was estimated in every voxel [24] and maps of fractional anisotropy (FA) and mean diffusivity (MD) were obtained. Subsequently, for each subject, average FA- and MD-values were calculated for the whole brain, which are indicators of microstructural integrity. FA measures the fraction of diffusion that is anisotropic, that is the fraction of water molecules moving in the direction of the axon, whereas MD measures the average motion of water molecules in all directions [25, 26]. To determine whether our group of older adults showed expected age-related neuronal changes, in addition to changes in gray and white matter volume as measured with structural MRI, we examined differences in whole-brain FA and MD between age groups using an independent t-test in SPSS.

5.3 Results

One older and two young adults were excluded from the data analyses because of anatomical abnormalities or artifacts. So, data from 15 young (age 25.5 ± 2.5 years) and 15 older adults (age 63.1 ± 5.2 years) were analyzed. One older participant did not understand the instructions for the motor task at the pre-test. Therefore, for this participant, we applied mean substitution for the pre-test motor performance values and only included the fMRI data of the post-test and retention test. Table 1 shows that participants' characteristics do not differ between the two age groups, except for age ($t_{20.026} = -25.28$, $p < 0.001$).

5.3.1 Behavioral results

Fig. 2 shows the motor performance in the experimental and control condition at the three time-points in the two age groups and Table 2 summarizes the absolute and percent changes in performance. A main effect of time ($F_{2, 56} = 59.8$, $p < 0.001$) showed that, averaged across age

groups and conditions, motor performance increased by 22% from pre- to post-test and by an additional 11% from post-test to retention test. The age ($F_{1, 28}$ = 12.8, p = 0.001) and condition ($F_{11, 28}$ = 135.9, p < 0.001) main effects showed that older compared with younger adults' motor performance was 3.2° (53%) worse and that, overall, participants performed 3.5° (37%) better at the control compared with the experimental condition. There were no age × time ($F_{2, 56}$ = 0.2, p = 0.790) or age × condition × time ($F_{2, 56}$ = 0.1, p = 0.929) interactions, indicating that both age groups improved their motor performance in both conditions at similar rates.

Table 1. Participants' characteristics.

Variable	Young adults	Older adults	Between-group difference	
	(n=15)	(n=15)	Test statistic	p-value
Age (y)	25.5 (2.5)	63.1 (5.2)	$t_{20.026}$ = -25.28	< 0.001
Sex (M/F)	6/9	9/6	χ^2_1 = 1.20	0.273
Height (m)	172.3 (9.5)	167.6 (12.1)	t_{28} = 1.18	0.249
Mass (kg)	68.5 (16.8)	71.3 (15.0)	t_{28} = -0.48	0.635
BMI (kg/m^2)	22.8 (3.6)	25.2 (3.7)	t_{28} = -1.84	0.076
Laterality quotient	0.71 (0.17)	0.78 (0.17)	t_{28} = -1.10	0.282
PSQI	4.4 (2.0)	4.5 (2.4)	t_{28} = -0.08	0.935
Quantity of sleep (h)	6.8 (1.2)	6.8 (1.5)	Mann-Whitney U = 99.5	0.584
Quality of sleep[a]	1	1	Mann-Whitney U = 111.0	0.936
MMSE score	-	29.2 (0.8)	-	-
GARS	-	18.9 (2.5)	-	-

Values are mean (±SD). Key: BMI, body mass index; GARS, Groningen Activity Restriction Scale (18–72, the higher the score, the higher the activity restriction); MMSE, Mini Mental State Examination (>27 cognitively healthy); PSQI, Pittsburgh Sleep Quality Index (lower score is higher quality of sleep in last month); [a] Median instead of mean, 4-point Likert scale, with values between 0 and 3, denoting high and poor quality of sleep in the night before retention testing, respectively.

Table 2. Motor performance improvements relative to pre-test performance.

		Absolute improvement (°)		Percent improvement (%)	
Condition	**Group**	Pre- to post-test	Pre-test to retention	Pre- to post-test	Pre-test to retention
Experimental	Older	3.30 (2.09)	3.39 (2.18)	25.0 (12.5)	25.7 (16.4)
	Young	2.51 (1.34)	3.04 (1.76)	25.8 (11.3)	31.3 (12.9)
Control	Older	1.48 (1.62)	2.95 (1.73)	16.3 (18.6)	32.6 (15.4)
	Young	0.91 (1.38)	1.98 (1.33)	17.5 (26.2)	38.2 (14.1)

Note: positive improvements reflect an increase in motor performance. Values represent mean (SD). There were main effects of age, condition and time (see section 5.3.1).

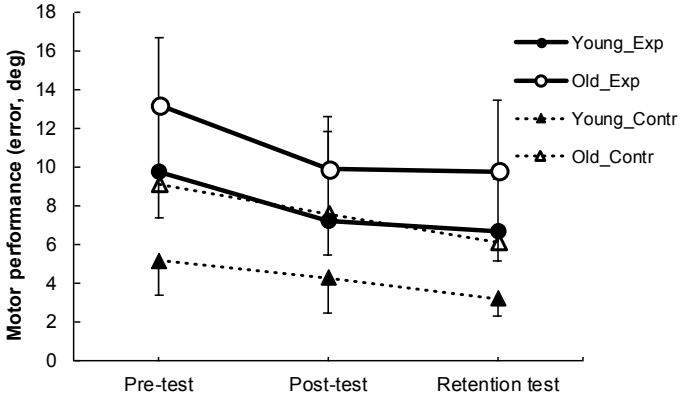

Fig. 2 Motor performance of young (filled symbols) and older adults (open symbols) on the experimental (solid line, circles) and control condition (dashed line, triangles). Motor performance is shown as mean error from the template in degrees. There were main effects of age, time and condition (see section 5.3.1).

5.3.2 Structural MRI and DTI results

Older adults had a smaller gray matter volume (older: 628 ± 57 ml; young: 741 ± 59 ml; $t_{28} = 5.3$, $p < 0.001$) and a higher cerebrospinal fluid volume (older: 322 ± 63 ml; young: 230 ± 51 ml; $t_{28} = -4.4$, $p < 0.001$) compared to young adults. There were no age-related differences in white matter volume (older: 427 ± 47 ml; young: 444 ± 46 ml; $t_{28} = 1.0$, $p = 0.325$).

DTI results revealed that older adults had, averaged across the whole brain, a lower FA (older: 0.18 ± 0.03; young: 0.22 ± 0.02; $t_{28} = 3.8$, $p = 0.001$) but similar MD (older: $1.04*10^{-9} \pm 0.19*10^{-9}$; young: $0.99*10^{-9} \pm 0.10*10^{-9}$; $t_{22.1} = -0.9$, $p = 0.363$) when compared to young adults.

5.3.3 fMRI results

5.3.3.1 Comparison of BOLD-signal between age groups and conditions

The first model revealed that older compared with young adults showed greater brain activation in a wide range of brain areas (Fig. 3), including the striatum, thalamus and hippocampus, pre- and post-central gyri, frontal, temporal and occipital/parietal areas bilaterally. We examined this effect in more detail by inspecting the activation patterns during each condition at each time point. This demonstrated that in a minority of these areas this effect seems to be related to greater deactivations in young compared to older adults, including the left insular cortex, frontal areas, precuneus, calcarine cortex, and fusiform gyrus; right rolandic operculum, precentral gyrus and amygdala; and bilateral hippocampus. In the majority of the areas, however, the results were due to higher brain activations in older compared with younger adults.

Furthermore, this model showed that there was greater activation when executing the experimental compared with the control condition in bilateral motor, parietal and occipital areas and cerebellum (Fig. 4, red/yellow blobs). However, during execution of the control compared with experimental condition, activation was greater in right middle frontal gyrus and right inferior parietal/angular gyrus (Fig. 4, blue/green blobs).

When whole-brain gray matter volume was added to the first model as a covariate, all regions (with the exception of the left hippocampus) that were greater activated in the older versus young adults, were no longer significant. This indicates that age-related differences in gray matter partially explained the age-related differences in brain activation. Additionally, the right supramarginal/post-central gyrus resulted to be significantly more activated in older compared with young adults only when entering the gray matter volume as a covariate to the model.

5.3.3.2 Effect of time on BOLD-signal

The second model took into consideration any time-related effects specific for the experimental condition. There was a main effect of time (Table 3) and post-hoc contrasts revealed that, across age groups, brain activation decreased from pre- to post-test in the parietal and occipital areas bilaterally, and increased back to pre-test levels from post-test to retention (Fig. 5, Supplementary materials: Table S1). Additionally, brain activation increased also from post-test to retention in the right superior/middle frontal gyrus.

5.3.3.3 Age by time interaction effect on BOLD-signal

The second model also revealed an age × time interaction, showing age-related differences in brain activation changes over time in the bilateral precuneus/posterior cingulum, left middle temporal gyrus, left inferior frontal gyrus, and left middle occipital/angular/middle temporal gyrus (Table 3, Fig. 6 left). Post-hoc contrasts showed that from post-test to retention, activation in the left inferior frontal gyrus increased in older adults but tended to decrease in young adults (Fig. 6 right, Supplementary materials: Table S2). However, in the other areas, activation decreased in young but tended to increase older adults (Fig 6. right, Supplementary materials: Table S2). When examining these results in more detail by extracting the parameter estimates of the GLM, it appeared that during both experimental and control condition and in both age groups, there were deactivations in all clusters (see Supplementary materials: Fig. S1). This indicates that there was less brain activation in these clusters during task execution compared with the rest condition at each time point. Changes in deactivation from post-test to retention occurred only when executing the experimental condition, while no changes occurred when performing the control condition. Hence, there were greater deactivations from post-test to retention in bilateral precuneus/posterior cingulum, left middle temporal gyrus and left middle occipital/angular/middle temporal gyrus during the experimental condition in young adults but there were no significant changes in these areas in older adults. Simultaneously, there was a trend for a smaller deactivation from post-test to retention in left inferior frontal gyrus in the older adults but no significant change was observed in the young adults. To summarize, while executing the experimental condition, from post-test to retention, there were trends for greater deactivations in young but smaller deactivations in older adults in bilateral precuneus, and left frontal, temporal, and occipital areas.

When whole-brain gray matter volume was added to the model as a covariate, the left middle temporal gyrus cluster from the age × time interaction of the second model was no longer significant, indicating that age-related differences in whole-brain gray matter volume partially explained the functional neural changes in this area after motor learning. There was no influence of gray matter volume on brain deactivation changes in the other clusters of the age × time interaction or time main effect.

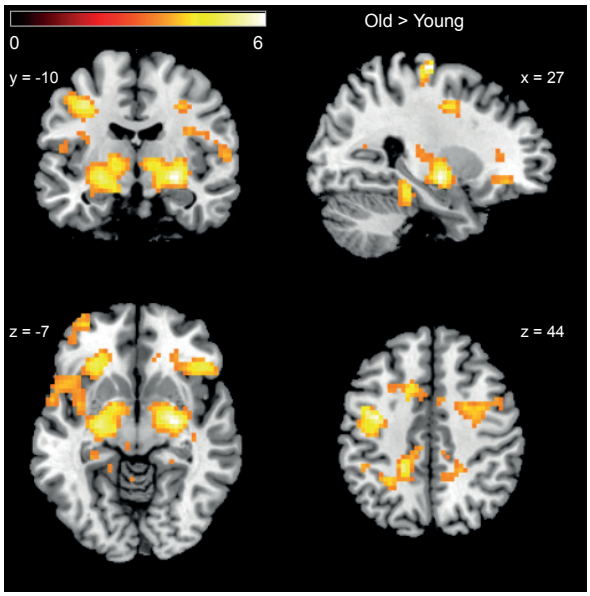

Fig. 3 Greater brain activation in older compared with young adults during the execution of the motor tasks (Z-scores). There were no regions with greater activation in young vs. older adults.

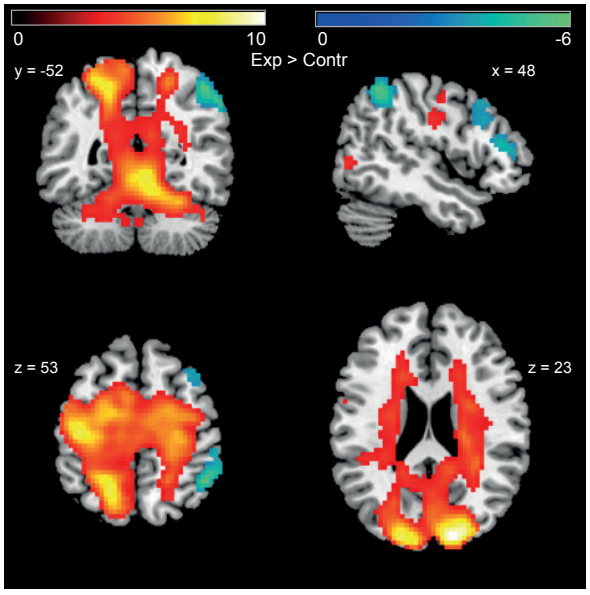

Fig. 4 Differences in brain activation between experimental and control condition, averaged across age-groups and time points (Z-scores). Red/yellow indicate greater activation in experimental condition compared with control condition, and blue/green indicate greater activation in control condition compared with experimental condition.).

5.4 Discussion

We examined age-related changes in brain activation after acquisition and consolidation (24-hours) of a visuomotor tracking skill. Young and older adults learned the skill to a similar extent and both age groups decreased brain activation in parietal and occipital areas bilaterally after skill acquisition. On the other hand, they increased activation in these same areas and in the right frontal cortex after motor memory consolidation. Older adults showed in general greater brain activation while executing the task. In contrast to brain activation, changes in brain deactivation

were age-dependent after consolidating the motor skill into motor memory. Young adults showed greater deactivations from post-test to retention in the bilateral precuneus and left occipital and temporal areas, whereas older adults showed smaller deactivations in the left inferior frontal area. These results suggest that older adults use an alternative strategy compared with young adults while learning a visuomotor tracking skill, which might be a compensatory mechanism for age-related structural changes.

Table 3. Effects of time and interaction between age and time on BOLD-signal.

Cluster(s)	Side	Peak voxel MNI coordinates			Cluster size (nr of voxels)	F-value
		X	Y	Z		
Time main effect						
Inferior frontal gyrus pars opercularis	L	-36	14	20	57	11.72
Superior occipital gyrus	L	-27	-73	23	68	12.16
Superior/middle occipital gyrus, precuneus, angular gyrus, middle temporal gyrus	R	21	-61	44	92	11.46
Superior/inferior parietal gyrus, precuneus, middle occipital gyrus	L	-18	-67	59	108	12.05
Middle frontal gyrus	R	33	11	59	33	11.05
Precuneus	L	-9	-58	62	31	11.97
Age × time interaction						
Middle temporal gyrus	L	-63	-40	2	30	11.07
Inferior frontal gyrus pars triangularis	L	-54	26	14	41	12.86
Middle occipital gyrus, angular gyrus, middle temporal gyrus	L	-39	-70	32	69	11.46
Precuneus, posterior cingulum	L+R	-3	-55	17	229	14.40

Note: This model included fMRI images of Experimental > Control contrasted images for both age-groups at all time-points as input.

5.4.1 Learning rate is similar in older and young adults

Although older adults performed worse on the visuomotor task compared with young adults, the practice-induced improvements in performance were similar in the two age groups. This finding is consistent with previous studies, using similar tasks with the wrist [4], and index finger [5]. An age-related decline in motor performance can be explained by deteriorations in nervous and neuromuscular systems with increasing age (for a review see [6]). The similar learning rates in young and older adults together with the hypothesized age-related differences in brain function suggest that alternative learning strategies might occur in the older brain to compensate for age-related declines in brain structure. This will be discussed in the next sections.

5.4.2 Structural declines occur in the aging brain
As expected, we found age-related structural declines in the brain. Older compared with younger adults had smaller gray matter volumes, increased cerebrospinal fluid volumes, and lower white matter anisotropy (FA). This is in agreement with previous studies [1, 18] and indicates that our older participants are probably a representative sample of the Italian healthy aging population.

5.4.3 Older adults show greater brain activation when executing a visuomotor tracking task
In agreement with many studies using motor and cognitive tasks (for reviews see [13, 27]) older compared with young adults showed greater brain activation when executing the visuomotor tracking task. This greater brain activation was shown in a wide range of brain areas, including bilateral striatum, thalamus and hippocampus, sensorimotor cortices, frontal, temporal, parietal and occipital areas. In some of these areas this effect seemed to be related to greater deactivations in young compared with older adults, including the left insular cortex, frontal areas, precuneus, calcarine cortex, and fusiform gyrus; right rolandic operculum, precentral gyrus and amygdala; and bilateral hippocampus. These results suggest that older adults rely more on striatal, thalamic, sensorimotor and temporal functions than young adults do, which could be an attempt to compensate for age-related structural declines (see section 5.3.2). The greater activation in older adults agrees with the CRUNCH model [13]. However, this compensatory strategy is only partially successful since the learning rate is similar between the age groups but the performance level of older adults is 3.2° (53%) worse when compared to that of young adults. Greater striatal activation in older compared with young adults is in agreement with some [28, 29] but not with other studies [30, 31]. The striatum is involved in feedback and decision-making [32, 33]. We argue that the greater striatal activation we observed in older compared with young individuals might be due to their poorer performance, as demonstrated by their higher error. Finally, the result that parts of the frontal, occipital and parietal cortices are less deactivated in older adults is in agreement with the idea that with advancing age there is a dysregulation of the default mode network (DMN; [34]).

To summarize, it seems that by over-activating cortical and subcortical motor areas, older adults rely on compensatory strategies. Though, this interpretation warrants some caution because age-related respiratory or vascular differences could have confounded the results [35, 36]. In other words, BOLD-signal is an indirect measure of neural activity, which is also influenced by cerebral blood flow, cerebral blood volume and cerebral blood oxygen consumption that are affected by age [36, 37]. Furthermore, age-related increases in muscle (co-)activation [38, 39] might also explain the greater brain activation in motor areas in older compared with younger adults. However, since we did not measure muscle activity, we cannot deduce this based on our data. Finally, the exact relationship between motor performance and brain activity is unclear. One possibility is that such a relationship is non-linear. Furthermore, increased brain activation could be inherent to a lower performance without necessarily indicating a compensatory strategy.

5.4.4 No age-related differences were demonstrated in brain activation changes
In contrast to our hypothesis [8, 14], brain activation was modulated similarly over time in young and older adults. More specifically, both age groups showed a decrease of brain activation from pre- to post-test in bilateral parietal and occipital areas and increased activation in these same areas back to pre-test levels from post-test to retention. Additionally, brain activation increased also from

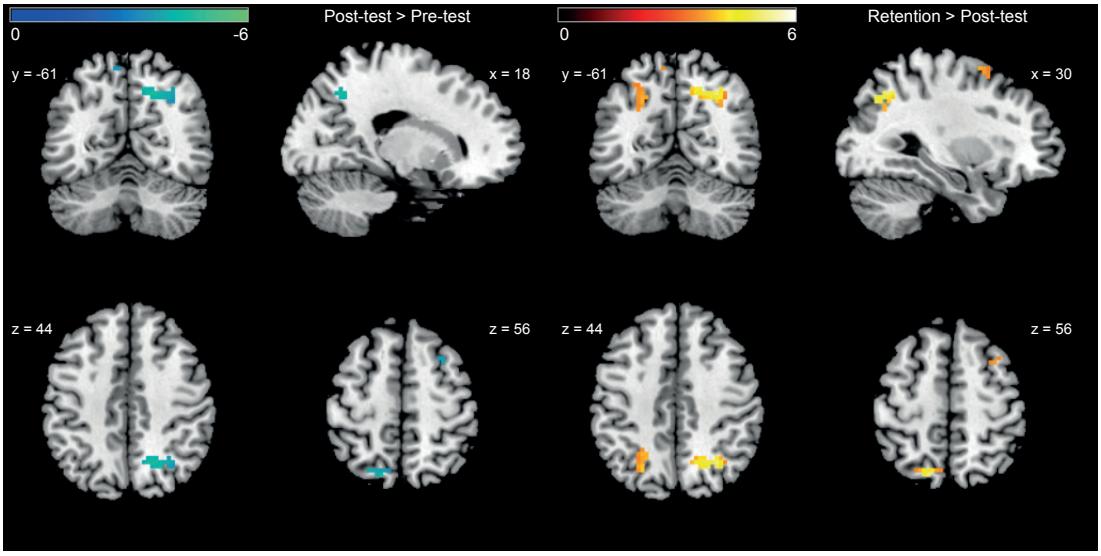

Fig. 5 Changes in brain activation from pre- to post-test (left) and post-test to retention test (right), averaged across age-groups (Z-scores). Blue/green indicate decreases and red/yellow indicate increases in brain activation over time. Experimental > Control contrasted images were used as input images in the statistical model.

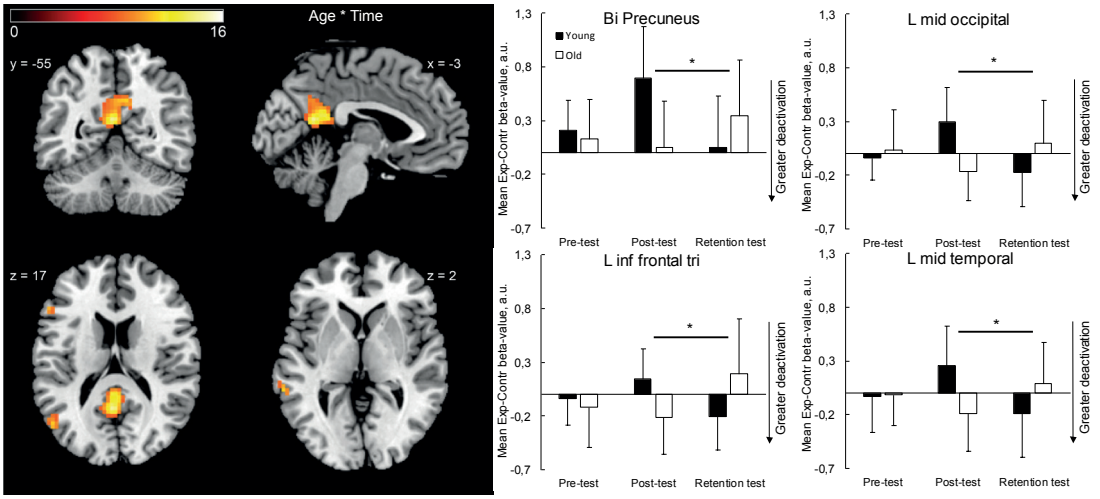

Fig. 6 Left side: Interaction of age × time on BOLD-signal (F-values). Experimental > control contrasted images were used as input images in the statistical model. Right side: Mean parameter estimates in the regions of the age ×time interaction effect (see left side) for experimental > control condition in both age groups. In each region, there was an interaction effect between young and older adults in changes in BOLD-signal from post-test to retention. An asterisk indicates a significant interaction between the two designated time points and the two age groups as determined by post-hoc BOLD-contrasts and corresponds to z-values <-4.

post-test to retention in the right frontal areas. These results suggest that visual processing areas are more involved when performing the visuomotor task for the first time and after a 24-hour offline period when compared to immediately after a training session. Our results agree with previous studies in young adults demonstrating that brain activation decreased when participants become more familiar with a task [40, 41]. Such a reduction may reflect more efficient signal processing after motor practice. Consistent with this interpretation, a recent magnetoencephalography study showed decreased beta event-related desynchronization in occipital cortices in participants performing an isometric ankle plantarflexion target matching task [42]. The novelty of the current study is the demonstration of more efficient visuospatial processing after a single motor training session in older adults. Our results are in line with the results by Santos Monteiro et al. (2017) [30] who showed that after 2 weeks of motor training brain activation changed similarly over time in young and older adults. However, they demonstrated decreased activation in left temporal, bilateral frontal and right thalamic areas, whereas we found decreased activation in bilateral occipital and parietal areas.

After 24 hours, brain activation increased back to pre-test levels in both age groups. This may indicate that approximately half an hour of task experience (at Day 1) may not be sufficient to retain the visuospatial processing efficiency a day later. Increased brain activation after motor memory consolidation including a night of sleep is in agreement with the limited research available in young adults, which showed increased activation in bilateral basal ganglia, bilateral temporal, left frontal, and cerebellar areas [43]. Based on age-related reductions in sleep spindle oscillations [8, 44], which most likely play an important role in the consolidation of newly acquired motor skills, we expected to find age-related differences in brain activation changes after an offline period. However, we found no such differences. Perhaps this is because there were no age-related behavioral differences after the 24-hour offline period, indicating that the motor skill was consolidated similarly in young and old adults. Additional research using a variety of motor tasks with additional experimental manipulations is required to further examine whether brain activation changes after an offline period involving a night of sleep are age-dependent.

5.4.5 There are age-related differences in brain deactivation changes

In contrast to changes in brain activation, changes in brain deactivation were age-dependent. After the motor memory consolidation phase, deactivation increased (i.e., greater deactivation) in young adults in bilateral precuneus and left occipital/angular and temporal areas and deactivation tended to increase in left inferior frontal cortex (<10 voxels). However, older adults decreased or tended to decrease brain deactivation in all of these areas (i.e., smaller deactivation). As expected, we found age-related differences in frontal and temporal areas. However, contrary to previous findings using motor sequence learning [8], our study demonstrated effects of age on changes in deactivations instead of activations after the offline period. Our results could be explained by the fact that the precuneus, angular and temporal area are part of the DMN [45, 46]. Interestingly, this modulation of deactivation is age-dependent and occurs only after motor memory consolidation. Apparently, older adults do not modulate the DMN in order to consolidate and retain the skill, which is in agreement with the idea that DMN modulation is dysregulated with increasing age [34]. Perhaps activating brain areas to a greater extent as shown in the older > young effect

(see section 5.4.3) is a possible mechanism of compensation.

5.4.6 Limitations

One limitation is that, sporadically, noise occurred in the wrist position signal of the manipulandum. In the behavioral data, we used a second order low-pass Butterworth filter of 5 Hz to account for this noise. In the second model of the fMRI analyses, examining the effects of time and the age × time interaction, we subtracted the brain activation during the control condition from the brain activation during the experimental condition. We believe that the noise in the manipulandum signal had no or minimal influences on these results since any brain activation that might be related to the occurrence of the noise in the manipulandum signal occurred in both experimental and control conditions and would therefore be filtered from the data. However, the comparison of brain activation between young and older adults in the first model should be taken with some caution since the noise occurred more often in older compared with young adults and we did not contrast Experimental > Control in this model. Another limitation is that not all participants participated in the study at similar times of the day. These diurnal variations could have affected neuroplasticity [47]. A final limitation was that our fMRI volumes did not completely cover the cerebellum. Since the cerebellum is known to be involved in motor learning [8, 14, 48, 49] and eye-hand coordination [50], our results could have underestimated the role of the cerebellum.

5.5 Conclusions

Age-related changes in brain activation after acquiring and consolidating a visuomotor tracking skill were examined. While there were age-related impairments in motor performance, older adults learned the skill as well as young adults. Changes in parietal and occipital activation, independent of age, suggest changes in visuospatial processing efficiency throughout the stages of motor learning. Finally, age-related deteriorations in modulating the activity of areas of the DMN after motor memory consolidation suggest that older adults use compensatory mechanisms to achieve similar learning rates as young adults. Presumably, this is achieved by activating brain areas to a greater extent during motor task execution.

References

1. Coupe P, Catheline G, Lanuza E, Manjon JV, Alzheimer's Disease Neuroimaging Initiative. Towards a unified analysis of brain maturation and aging across the entire lifespan: A MRI analysis. Hum Brain Mapp. 2017;38(11):5501-18.

2. Salat DH, Tuch DS, Greve DN, van der Kouwe AJ, Hevelone ND, Zaleta AK, et al. Age-related alterations in white matter microstructure measured by diffusion tensor imaging. Neurobiol Aging. 2005;26(8):1215-27.

3. Lehmann K, Steinecke A, Bolz J. GABA through the ages: Regulation of cortical function and plasticity by inhibitory interneurons. Neural Plast. 2012;2012.

4. Berghuis K, De Rond V, Zijdewind I, Koch G, Veldman M, Hortobágyi T. Neuronal mechanisms of motor learning are age-dependent. Neurobiol Aging. 2016;46:149-59.

5. Cirillo J, Todd G, Semmler JG. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. Eur J Neurosci. 2011;34(11):1847-56.

6. Seidler RD, Bernard JA, Burutolu TB, Fling BW, Gordon MT, Gwin JT, et al. Motor control and aging: Links to age-related brain structural, functional, and biochemical effects. Neurosci Biobehav Rev. 2010;34(5):721-33.

7. Dayan E, Cohen LG. Neuroplasticity subserving motor skill learning. Neuron. 2011;72(3):443-54.

8. Fogel SM, Albouy G, Vien C, Popovicci R, King BR, Hoge R, et al. fMRI and sleep correlates of the age-related impairment in motor memory consolidation. Hum Brain Mapp. 2014;35(8):3625-45.

9. Lin CH, Chiang MC, Wu AD, Iacoboni M, Udompholkul P, Yazdanshenas O, et al. Age related differences in the neural substrates of motor sequence learning after interleaved and repetitive practice. Neuroimage. 2012;62(3):2007-20.

10. Cabeza R. Hemispheric asymmetry reduction in older adults: The HAROLD model. Psychol Aging. 2002;17(1):85.

11. Cabeza R, Anderson ND, Locantore JK, McIntosh AR. Aging gracefully: Compensatory brain activity in high-performing older adults. Neuroimage. 2002;17(3):1394-402.

12. Anguera JA, Reuter-Lorenz PA, Willingham DT, Seidler RD. Failure to engage spatial working memory contributes to age-related declines in visuomotor learning. J Cogn Neurosci. 2011;23(1):11-25.

13. Reuter-Lorenz PA, Cappell KA. Neurocognitive aging and the compensation hypothesis. Current Directions in Psychological Science. 2008;17(3):177-82.

14. Rieckmann A, Fischer H, Backman L. Activation in striatum and medial temporal lobe during sequence learning in younger and older adults: Relations to performance. Neuroimage. 2010;50(3):1303-12.

15. Oldfield RC. The assessment and analysis of handedness: The edinburgh inventory. Neuropsychologia. 1971;9(1):97-113.

16. Kempen GI, Miedema I, Ormel J, Molenaar W. The assessment of disability with the groningen activity restriction scale. conceptual framework and psychometric properties. Soc Sci Med. 1996;43(11):1601-10.

17. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12(3):189-98.

18. Sullivan EV, Pfefferbaum A. Neuroradiological characterization of normal adult ageing. Br J Radiol. 2007;80 Spec No 2:S99-108.

19. Toxopeus CM, de Jong BM, Valsan G, Conway BA, Leenders KL, Maurits NM. Direction of movement is encoded in the human primary motor cortex. PLoS One. 2011;6(11):e27838.

20. Berghuis KM, Veldman MP, Solnik S, Koch G, Zijdewind I, Hortobagyi T. Neuronal mechanisms of motor learning and motor memory consolidation in healthy old adults. Age. 2015;37:53.

21. Savalia NK, Agres PF, Chan MY, Feczko EJ, Kennedy KM, Wig GS. Motion-related artifacts in structural brain images revealed with independent estimates of in-scanner head motion. Hum Brain Mapp. 2017;38(1):472-92.

22. Chen G, Saad ZS, Britton JC, Pine DS, Cox RW. Linear mixed-effects modeling approach to FMRI group analysis. Neuroimage. 2013;73:176-90.

23. Leemans A, Jones DK. The B-matrix must be rotated when correcting for subject motion in DTI data. Magn Reson Med. 2009;61(6):1336-49.

24. Basser PJ, Mattiello J, LeBihan D. Estimation of the effective self-diffusion tensor from the NMR spin echo. J Magn Reson B. 1994;103(3):247-54.

25. Basser PJ. Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. NMR Biomed. 1995;8(7-8):333-44.

26. Pierpaoli C, Jezzard P, Basser PJ, Barnett A, Di Chiro G. Diffusion tensor MR imaging of the human brain. Radiology. 1996;201(3):637-48.

27. Ward NS. Compensatory mechanisms in the aging motor system. Ageing Res Rev. 2006;5(3):239-54.

28. Mattay VS, Fera F, Tessitore A, Hariri AR, Das S, Callicott JH, et al. Neurophysiological correlates of age-related changes in human motor function. Neurology. 2002;58(4):630-5.

29. Ward NS, Swayne OB, Newton JM. Age-dependent changes in the neural correlates of force modulation: An fMRI study. Neurobiol Aging. 2008;29(9):1434-46.

30. Santos Monteiro T, Beets IAM, Boisgontier MP, Gooijers J, Pauwels L, Chalavi S, et al. Relative cortico-subcortical shift in brain activity but preserved training-induced neural modulation in older adults during bimanual motor learning. Neurobiol Aging. 2017;58:54-67.

31. Van Impe A, Coxon JP, Goble DJ, Wenderoth N, Swinnen SP. Ipsilateral coordination at preferred rate: Effects of age, body side and task complexity. Neuroimage. 2009;47(4):1854-62.

32. Balleine BW, Delgado MR, Hikosaka O. The role of the dorsal striatum in reward and decision-making. J Neurosci. 2007;27(31):8161-5.

33. Hiebert NM, Vo A, Hampshire A, Owen AM, Seergobin KN, MacDonald PA. Striatum in stimulus-response learning via feedback and in decision making. Neuroimage. 2014;101:448-57.

34. Park DC, Reuter-Lorenz P. The adaptive brain: Aging and neurocognitive scaffolding. Annu Rev Psychol. 2009;60:173-96.

35. Kannurpatti SS, Motes MA, Rypma B, Biswal BB. Neural and vascular variability and the fMRI-BOLD response in normal aging. Magn Reson Imaging. 2010;28(4):466-76.

36. D'Esposito M, Deouell LY, Gazzaley A. Alterations in the BOLD fMRI signal with ageing and disease: A challenge for neuroimaging. Nat Rev Neurosci. 2003;4(11):863-72.

37. Leenders KL, Perani D, Lammertsma AA, Heather JD, Buckingham P, Healy MJ, et al. Cerebral blood flow, blood volume and oxygen utilization. normal values and effect of age. Brain. 1990;113(Pt 1):27-47.

38. Hortobagyi T, Devita P. Mechanisms responsible for the age-associated increase in coactivation of antagonist muscles. Exerc Sport Sci Rev. 2006;34(1):29-35.

39. Rozand V, Senefeld JW, Hassanlouei H, Hunter SK. Voluntary activation and variability during maximal dynamic contractions with aging. Eur J Appl Physiol. 2017;117(12):2493-507.

40. Boe SG, Cassidy RJ, McIlroy WE, Graham SJ. Single session motor learning demonstrated using a visuomotor task: Evidence from fMRI and behavioural analysis. J Neurosci Methods. 2012;209(2):308-19.

41. Gobel EW, Parrish TB, Reber PJ. Neural correlates of skill acquisition: Decreased cortical activity during a serial interception sequence learning task. Neuroimage. 2011;58(4):1150-7.

42. Gehringer JE, Arpin DJ, Heinrichs-Graham E, Wilson TW, Kurz MJ. Neurophysiological changes in the visuomotor network after practicing a motor task. J Neurophysiol. 2018;120(1):239-49.

43. Debas K, Carrier J, Orban P, Barakat M, Lungu O, Vandewalle G, et al. Brain plasticity related to the consolidation of motor sequence learning and motor adaptation. Proc Natl Acad Sci U S A. 2010;107(41):17839-44.

44. Peters KR, Ray L, Smith V, Smith C. Changes in the density of stage 2 sleep spindles following motor learning in young and older adults. J Sleep Res. 2008;17(1):23-33.

45. Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. A default mode of brain function. Proc Natl Acad Sci U S A. 2001;98(2):676-82.

46. Raichle ME. The brain's default mode network. Annu Rev Neurosci. 2015;38:433-47.

47. Sale MV, Ridding MC, Nordstrom MA. Cortisol inhibits neuroplasticity induction in human motor cortex. J Neurosci. 2008;28(33):8285-93.

48. Daselaar SM, Rombouts SA, Veltman DJ, Raaijmakers JG, Jonker C. Similar network activated by young and old adults during the acquisition of a motor sequence. Neurobiol Aging. 2003;24(7):1013-9.

49. Lefebvre S, Dricot L, Gradkowski W, Laloux P, Vandermeeren Y. Brain activations underlying different patterns of performance improvement during early motor skill learning. Neuroimage. 2012;62(1):290-9.

50. Miall RC, Reckess GZ, Imamizu H. The cerebellum coordinates eye and hand tracking movements. Nat Neurosci. 2001;4(6):638-44.

Supplementary materials

Table S1. Post-hoc effects of the Time main effect on BOLD-signal

Cluster(s)	Side	Peak voxel MNI coordinates			Cluster size (nr of voxels)	Z-value
		X	Y	Z		
Post-test > Pre-test						
Precuneus, superior/middle occipital gyrus, angular gyrus	R	18	-61	44	54	-4.54
Superior occipital gyrus	L	-24	-76	26	2	-3.35
Middle occipital gyrus	L	-33	-70	29	1	-3.38
	R	30	-64	32	1	-3.29
Superior parietal gyrus, precuneus	L	-15	-70	56	11	-4.21
Middle frontal gyrus	R	30	11	56	4	-3.73
	R	33	14	59	1	-3.50
Precuneus	L	-9	-55	62	15	-4.09
	L+R	0	-58	62	1	-3.47
Retention > Post-test						
Angular gyrus, superior/middle occipital gyrus, precuneus	R	30	-61	44	81	5.12
Superior/middle occipital gyrus	L	-30	-67	29	9	3.46
Inferior frontal gyrus pars opercularis, precentral gyrus	L	-45	5	26	9	3.73
Superior/inferior parietal gyrus, middle occipital gyrus	L	-24	-55	50	65	4.37
Superior parietal gyrus, precuneus	L	-15	-70	56	15	5.14
Superior/middle frontal gyrus	R	30	5	62	13	3.78
Middle frontal gyrus	R	36	14	56	1	3.43
Precuneus	L	-6	-58	65	6	3.47
	L+R	0	-58	62	1	3.36
Retention > Pre-test						
Insula	L	-33	14	17	1	1.99

Table S2. Post-hoc effects of the Age × Time interaction on BOLD-signal

Cluster(s)	Side	Peak voxel MNI coordinates			Cluster size (nr of voxels)	Z-value
		X	Y	Z		
***Young*$_{Post>Pre}$ > *Old*$_{Post>Pre}$**						
Middle temporal gyrus	L	-51	-67	14	8	3.64
Precuneus	L	-3	-55	14	1	3.32
	R	6	-49	14	5	3.39
	R	6	-61	32	1	3.33
Middle occipital gyrus	L	-42	-67	26	2	3.40
	L	-39	-70	32	5	3.69
***Young*$_{Retention>Post}$ > *Old*$_{Retention>Post}$**						
Middle temporal gyrus	L	-63	-40	2	30	-4.70
Inferior frontal gyrus pars triangularis	L	-54	26	14	41	-4.93
Middle occipital gyrus, angular gyrus, middle temporal gyrus	L	-39	-67	32	69	-4.58
Precuneus, posterior cingulum, calcarine sulcus	L+R	-3	-55	17	229	-5.35
***Young*$_{Retention>Pre}$ > *Old*$_{Retention>Pre}$**						
Inferior frontal gyrus	L	-54	26	14	3	-3.48
Young: Post-test > Pre-test						
Precuneus, posterior cingulum, calcarine sulcus	L+R	-3	-58	17	95	4.22
Middle temporal gyrus	L	-51	-70	20	12	3.88
Young: Retention > Post-test						
Middle temporal gyrus	L	-60	-37	-7	21	-4.54
	L	-66	-34	-1	1	-3.32
Inferior frontal gyrus pars triangularis	L	-54	26	5	7	-3.73
Middle temporal gyrus, angular gyrus, middle occipital gyrus	L	-54	-64	17	53	-4.73
Precuneus, posterior cingulum, calcarine sulcus	L+R	-3	-55	14	210	-5.35
Young: Retention > Pre-test						
Inferior frontal gyrus	L	-54	26	5	1	-2.99

Table S2 Continued. Post-hoc effects of the Age × Time interaction on BOLD-signal

Cluster(s)	Side	Peak voxel MNI coordinates			Cluster size (nr of voxels)	Z-value
		X	Y	Z		
Old: Post-test > Pre-test						
Middle occipital gyrus	L	-36	-67	29	1	-3.49
	L	-36	-70	32	2	-4.05
Old: Retention > Post-test						
Middle temporal gyrus	L	-63	-40	2	1	3.39
	L	-48	-67	11	2	3.60
Inferior frontal gyrus pars triangularis	L	-54	29	14	20	4.07
Middle occipital gyrus	L	-36	-70	32	4	3.73
Precuneus	R	12	-55	29	2	3.42
Old: Retention > Pre-test						
Inferior frontal gyrus pars triangularis	L	-54	26	14	9	3.92

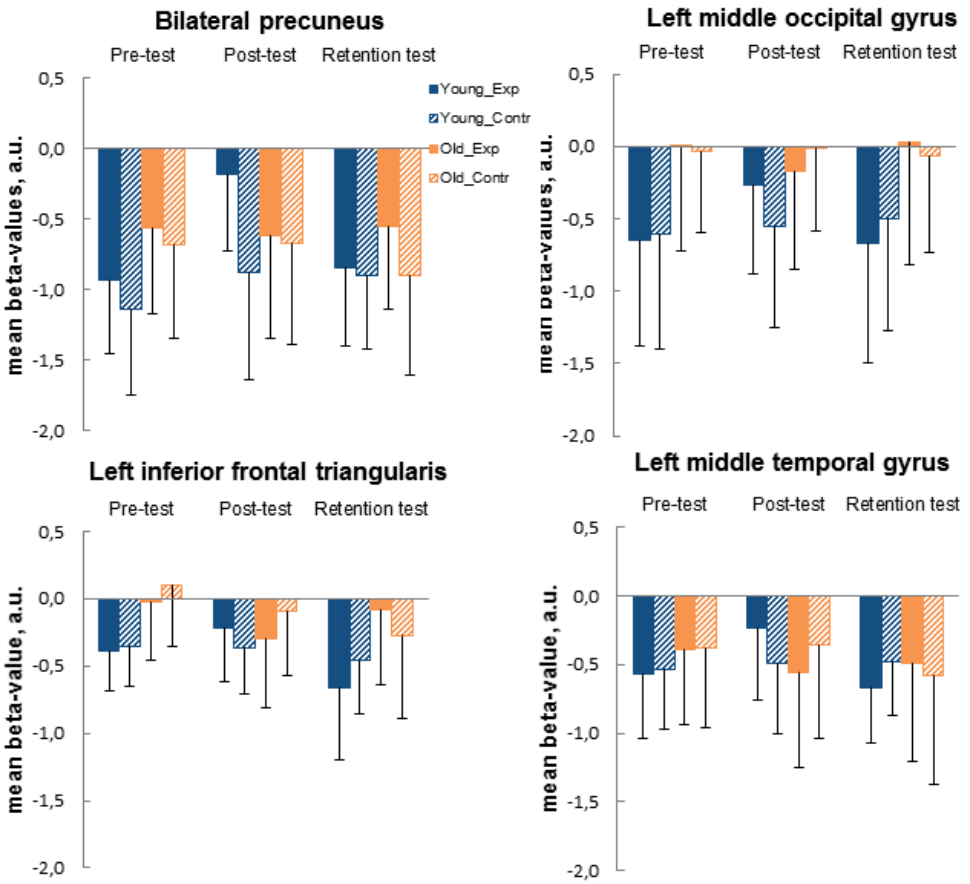

Fig. S1 Mean parameter estimates in the regions of the Age by Time interaction effect (see Fig 5) for experimental and control tasks separately in both age groups at each time moment. In both age groups, there were no significant changes over time in beta-values of the control task but beta-values of the experimental task decreased (greater deactivation) from post-test to retention test in young adults in bilateral precuneus, left middle occipital gyrus and left middle temporal gyrus.

| Chapter 6 |

General discussion

6.1 Main findings

Increasing age is accompanied by structural and functional changes in the peripheral and central nervous system, such as decreases in the number of motor units, a reduction in nerve conduction velocity, a decline in proprioception, reductions in gray and white matter volume, and increases in cerebrospinal fluid [1-5]. Despite these changes, older adults are still capable of learning new motor skills [6-8]. How do older adults sustain the ability to learn new motor skills? Do the neural mechanisms of motor learning change with advancing age, and if yes, how? Answers to these questions can contribute to improved neurorehabilitation protocols because neurostimulation settings might require adjustment to age. The aim of this thesis was therefore to examine the age-related differences in the underlying neural mechanisms of motor learning. We hypothesized that older adults would use alternative strategies of neural plasticity compared with young adults to learn new motor skills, perhaps to compensate for age-related structural and functional changes in the brain.

Non-invasive neurostimulation (transcranial magnetic stimulation, TMS; chapters 2 and 3) and neuroimaging (functional magnetic resonance imaging, fMRI; chapter 5) techniques were used to measure changes in neural mechanisms after the acquisition and motor memory consolidation phase. Furthermore, chapter 4 provides an overview of TMS studies examining motor learning in aging. The experimental chapters and the review chapter consistently show that visuomotor tracking performance is impaired in older compared with young adults but that the learning rate is similar across age groups (chapters 3, 4, and 5). These results suggest that older adults are capable of learning novel visuomotor skills but that older adults are unable to reach the same performance levels as that of young adults, or that older adults might need additional practice sessions to accomplish this. Both TMS and fMRI findings show that age-related differences in neural plasticity are mainly present after motor memory consolidation (chapters 3 and 5) but not necessarily or to a lesser extent after motor skill acquisition (chapters 3, 4, and 5). In this chapter, I will discuss the main findings of this thesis in more detail.

6.2 Age-related differences in neural plasticity after motor learning

6.2.1 No age-related differences in neural plasticity after motor skill acquisition

After a single training session, young and older adults acquired the visuomotor skill at similar rates (chapters 3, 4, and 5). In agreement with the long-term potentiation (LTP) hypothesis [9-11], CSE at rest increased after the acquisition period independent of age, evidenced by the pooled data in the meta-analysis (chapter 4). However, we were unable to demonstrate increases in CSE in our experimental chapters (chapters 2 and 3). In addition, SICI measured at rest decreased in older adults after visuomotor skill acquisition (chapters 2 and 4), indicating a reduction of gamma-aminobutyric acid-ergic (GABAergic) inhibition. However, this reduced inhibition effect was not seen in young adults when measured at rest (chapters 3 and 4) but only when measured during task execution (chapter 3). Our meta-analysis included only papers that had at least a group of older adults. Therefore, chapter 4 might not provide the best representation of neural plasticity occurring in young adults. Hence, this thesis provides an indication that similar plasticity mechanisms are involved in motor skill acquisition in older adults as to those previously demonstrated in young adults. Correspondingly, our fMRI results in chapter 5 also show similar neural mechanisms after

visuomotor skill acquisition in the two age groups, reflected by a decrease in parietal and occipital activation independent of age. These results collectively indicate that, in contrast to what was expected, older adults use similar strategies to acquire a visuomotor tracking skill. Perhaps, there is no need for older adults to utilize alternative strategies during this phase of motor learning. It could be that although older adults showed the expected age-related declines in gray matter volume and white matter integrity (chapter 5), the remaining resources might still be sufficient to acquire the visuomotor skill. An alternative explanation could be that older adults do not alter corticospinal and intracortical excitability modulation from pre- to post-practice but that they implement an alternative strategy while executing the task, as indicated by greater brain activation during task execution in older versus young adults (chapter 5). This age-effect is also demonstrated in other motor and cognitive tasks and is frequently related to better performance in older adults, hence interpreted as a compensation strategy (for reviews see [12, 13]). However, from the results in chapter 5, we cannot deduce whether this increased brain activation in older adults is compensatory, or whether it is a form of deterioration, because older adults' performance level is lower than that of young adults. To summarize, despite age-related structural (sub)cortical declines, there are no, or only small, age-related differences in neural plasticity after motor skill acquisition. The two age groups acquired the visuomotor skill at similar learning rates. It is suggested that the greater brain activation seen in older adults during task execution might be a compensatory strategy to accomplish these learning rates.

6.2.2 Age-related differences in neural plasticity after motor memory consolidation

In contrast to the retention of sequential motor skills and motor adaptation skills (for a review see [14]), young and older adults retained the acquired visuomotor tracking skill in the current thesis at similar rates after a 24-hour offline period (chapters 3 and 5). After the offline period, however, CSE measured both at rest and during the task and SICI measured during the task changed in opposite directions in the two age groups (chapter 3). More specifically, both measures of CSE increased in young but decreased in older adults. SICI values measured during task execution increased in older but decreased in young adults, resulting in a reduction of intracortical inhibition in older but an increase in intracortical inhibition in young adults after motor memory consolidation. Consistent with these findings, our fMRI experiment also revealed an opposite direction of change in the two age groups in deactivation after motor memory consolidation in the precuneus bilaterally, and in the left frontal, temporal, and occipital/angular areas (chapter 5), some of which are involved in the default mode network (DMN). Young adults tended to show greater deactivations in these areas after motor memory consolidation, whereas older adults tended to show smaller deactivations. According to a magnetic resonance spectroscopy and transcranial direct current stimulation (tDCS) study, greater brain activation in the sensorimotor cortex is related to reduced gamma-amino-butyric-acid (GABA) concentration in this area [15]. It could therefore be speculated that a reduction in SICI, which is known to be influenced by GABA-A receptor activation, could be related to increased brain activation or conversely to smaller deactivation. We indeed demonstrated a reduction in SICI measured during the task after motor memory consolidation in older adults (chapter 3), and smaller deactivations in default mode network regions from directly after practice to 24-hours later (chapter 5). Although these results are found in different networks, it is an interesting finding that changes in SICI in M1 and changes in deactivation in the DMN follow

the same pattern after motor memory consolidation in older adults. Contrary to our speculation, the study from Stagg et al. found a relationship between reduced GABA concentration induced by tDCS and decreases in M1 activity in healthy young adults [15]. However, it remains unknown how SICI relates to brain activation. Based on the results in this thesis, it is speculated that after a 24-hour consolidation period, older adults reduce intracortical inhibition not only in M1 but also in areas related to the default mode network but future studies are needed to investigate this hypothesis.

The finding that both TMS and fMRI results change in opposite directions after the consolidation period in the two age groups, while both age groups retained the acquired skill 24-hours after practice to similar rates, supports a compensatory strategy in older adults for age-related structural declines. The result that baseline-similar subgroups (chapter 3) also show age-related differences in neural plasticity after motor memory consolidation favor the idea that older adults use a compensatory strategy and that these neural changes are probably not merely due to lower performance levels. Another compensatory strategy might be the greater brain activation that was observed during task execution in older adults compared with young adults in chapter 5. However, because older adults' performance at the posttests and retention tests was worse compared to young adults (also in baseline-similar groups), there is no certainty whether the opposite TMS and fMRI changes in the two age groups are compensatory or whether they might be indicative of deterioration of the aging brain. Because of the similar learning rates in the two age groups, we favor the interpretation of a compensatory strategy in the older adults, but future studies are needed to examine this theory.

An important element influencing motor memory consolidation is sleep, more specifically sleep spindles, which mainly occur during stage 2 of sleep (for a review see [16]). Older adults suffer from disrupted sleep. Although sleep quality and quantity as measured by the Pittsburgh Sleep Quality Index did not differ in the two age groups in this thesis, older age is known to be associated with increased sleep fragmentation, decreased sleep time and efficiency, longer durations of sleep stages 1 and 2, and decreased amplitude, duration and number of sleep spindles (for reviews see [14, 16]). It is interesting that the age-related differences in neural mechanisms of motor learning in the current thesis are especially profound after motor memory consolidation and less after skill acquisition. It might be that during the motor memory consolidation phase, older adults not only need to compensate for age-related structural declines in the brain but also for age-related disruptions of sleep, in order to maintain the learning rate as high as young adults.

Fig. 1 shows a conceptual model that summarizes the main findings and interpretations discussed in this section. Taken together, structural and functional changes in the aging neuromuscular system require adaptive, perhaps compensatory strategies to learn new motor skills, especially during motor memory consolidation.

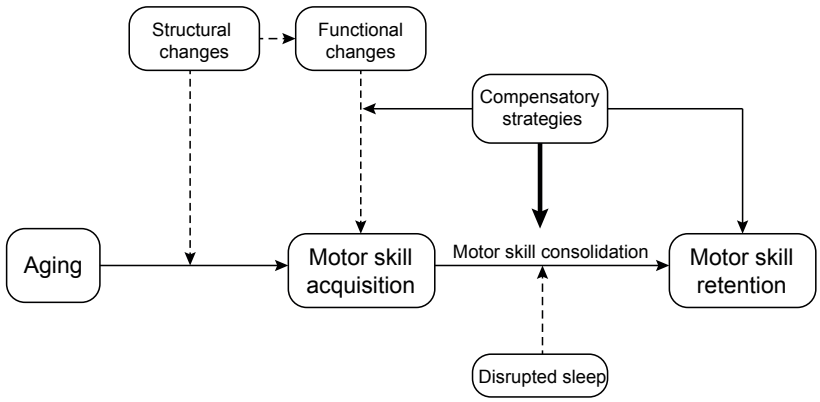

Fig. 1 Conceptual model that summarizes the main findings and interpretations of this thesis. Increasing age leads to structural and functional changes in the neuromuscular system, which can have a negative influence on motor learning. However, compensatory strategies such as greater brain activation or modulating corticospinal or intracortical processes differently than young adults might reduce or overcome some of these negative effects of age. Solid arrows indicate positive influences; thick solid arrow indicates a greater, and better substantiated positive influence; dashed arrows indicate negative influences.

6.3 Changes in corticospinal excitability and intracortical inhibition are not related to behavioral changes.

Although we found several strong correlations [17] between motor skill acquisition and CSE and SICI modulations in chapters 2 and 3, a meta-analysis combining individual data (old, n = 123; young, n = 128) compiled from 10 TMS studies in chapter 4 showed no relationship between neural plasticity measured at rest and behavioral changes in either age group. There might be several reasons for a lack of correlation between these variables (for reviews see [18, 19]). First, the relationship between changes in TMS metrics and behavioral changes might be non-linear. Second, motor-evoked potentials are influenced by excitability changes in the spinal motoneuron pool caused by corticospinal pathways originating from brain areas other than the stimulated M1. Finally, CSE consists not only of contributions of the stimulated M1 but also from inputs to M1, such as the premotor cortex and the supplementary motor area, which are not measured when TMS is applied at rest. Therefore, in chapters 2 and 3, we applied TMS during task execution, that is, when the brain is in the same "active" state as while learning the skill. However, this thesis does not confirm that CSE and SICI measured during the task provides a better insight into the underlying neuronal mechanisms of motor learning compared with measurements at rest. Since this was only a first attempt to measure TMS metrics during task execution, it might be that we used suboptimal settings. Future studies should examine which TMS settings are most effective for measuring CSE and SICI during muscle contraction or task execution (e.g. stimulation intensity, or level of muscle contraction).

Because changes in CSE and intracortical inhibition are unrelated to behavioral changes, the TMS results in this thesis should be taken with some caution. However, our fMRI results in chapter 5 agree with our TMS results (chapter 3) that neural plasticity after motor memory consolidation

differs between young and old adults (see section 6.2.2). Although our TMS and fMRI experiments reveal these age-related differences in different areas, this thesis gives a first indication of different motor memory consolidation strategies in young and older adults.

6.4 Limitations and future recommendations

This thesis has several limitations. First, both TMS and fMRI only provide an indirect measure of the underlying neural mechanisms of motor learning [20, 21]. Future studies are encouraged to add more direct ways of assessing brain activity, such as electroencephalography, or manipulate neural plasticity, using for example repetitive TMS, transcranial direct current stimulation, or paired associative stimulation, so that a more complete insight into the age-related differences in neural plasticity during motor learning can be obtained. Furthermore, network analyses can provide more insight into the neural mechanisms of motor learning because it includes communication between brain areas [22, 23]. Second, in all experimental chapters, we used a visuomotor tracking task executed with the wrist joint. Although this provides opportunities for comparison between chapters of this thesis, it complicates generalization of our results to other motor learning paradigms. As indicated in our systematic review and meta-analysis in chapter 4, the neural mechanisms of motor skill learning can be task-dependent. Third, in this thesis, we examined for the first time age-related changes in neural mechanisms after both visuomotor skill acquisition and after a 24-hour consolidation period, but the effects of age on the underlying mechanisms of long-term retention after weeks or months remain unknown. Because this thesis shows that changes in neural plasticity are mainly age-dependent after the consolidation period, future studies are recommended to include multiple retention tests. Finally, additional research should be performed to examine whether neurostimulation protocols should be adapted for older adults or patients given the age-related changes in neural plasticity after motor memory consolidation presented in this thesis.

6.5 Conclusions

This thesis contributes to our understanding of the age-related differences in neural plasticity after motor learning. We used non-invasive neurostimulation (TMS) and neuroimaging (fMRI) techniques to examine age-related changes in corticospinal and intracortical excitability, and brain activation and deactivation. New approaches we implemented in this thesis are that we not only applied TMS at rest but also during task execution, and we examined age-related differences in neural plasticity after both the skill acquisition and motor memory consolidation phase. Based on the results in this thesis we conclude that neural plasticity during visuomotor skill acquisition is similar in young and older adults. In contrast, while consolidating a newly acquired visuomotor tracking skill to similar rates as young adults, older adults use adaptive and perhaps compensatory neural strategies in order to retain the skill. Furthermore, TMS measures are not related to motor skill acquisition, emphasizing the need for the adaptation of research methods or application of combined neurostimulation and neuroimaging techniques to gain better insights into age-related differences in neural plasticity. We encourage neurorehabilitation programs including neurostimulation techniques to optimize the stimulation protocols to the age group and stage of motor learning.

References

1. Doherty TJ, Vandervoort AA, Brown WF. Effects of ageing on the motor unit: A brief review. Can J Appl Physiol. 1993;18(4):331-58.

2. Fjell AM, Walhovd KB. Structural brain changes in aging: Courses, causes and cognitive consequences. Rev Neurosci. 2010;21(3):187-221.

3. Li KY, Wu YH. Clinical evaluation of motion and position sense in the upper extremities of the elderly using motion analysis system. Clin Interv Aging. 2014;9:1123-31.

4. Rinderknecht MD, Lambercy O, Raible V, Liepert J, Gassert R. Age-based model for metacarpophalangeal joint proprioception in elderly. Clin Interv Aging. 2017;12:635-43.

5. Verdu E, Ceballos D, Vilches JJ, Navarro X. Influence of aging on peripheral nerve function and regeneration. J Peripher Nerv Syst. 2000;5(4):191-208.

6. Brown RM, Robertson EM, Press DZ. Sequence skill acquisition and off-line learning in normal aging. PLoS One. 2009;4(8):e6683.

7. Cirillo J, Rogasch NC, Semmler JG. Hemispheric differences in use-dependent corticomotor plasticity in young and old adults. Exp Brain Res. 2010;205(1):57-68.

8. Cirillo J, Todd G, Semmler JG. Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. Eur J Neurosci. 2011;34(11):1847-56.

9. Jensen JL, Marstrand PC, Nielsen JB. Motor skill training and strength training are associated with different plastic changes in the central nervous system. J Appl Physiol (1985). 2005;99(4):1558-68.

10. Perez MA, Lungholt BK, Nyborg K, Nielsen JB. Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. Exp Brain Res. 2004;159(2):197-205.

11. Ziemann U, Ilic TV, Pauli C, Meintzschel F, Ruge D. Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. J Neurosci. 2004;24(7):1666-72.

12. Reuter-Lorenz PA, Cappell KA. Neurocognitive aging and the compensation hypothesis. Current Directions in Psychological Science. 2008;17(3):177-82.

13. Ward NS. Compensatory mechanisms in the aging motor system. Ageing Res Rev. 2006;5(3):239-54.

14. King BR, Fogel SM, Albouy G, Doyon J. Neural correlates of the age-related changes in motor sequence learning and motor adaptation in older adults. Front Hum Neurosci. 2013;7:142.

15. Stagg CJ, Bachtiar V, Johansen-Berg H. The role of GABA in human motor learning. Curr Biol. 2011;21(6):480-4.

16. Fogel SM, Smith CT. The function of the sleep spindle: A physiological index of intelligence and a mechanism for sleep-dependent memory consolidation. Neurosci Biobehav Rev. 2011;35(5):1154-65.

17. Field A. Discovering statistics using SPSS. Sage publications; 2009.

18. Bestmann S, Krakauer JW. The uses and interpretations of the motor-evoked potential for understanding behaviour. Exp Brain Res. 2015;233(3):679-89.

19. Carson RG, Ruddy KL, McNickle E. What do TMS-evoked motor potentials tell us about motor learning? Adv Exp Med Biol. 2016;957:143-57.

20. Barker AT, Jalinous R, Freeston IL. Non-invasive magnetic stimulation of human motor cortex. Lancet. 1985;1(8437):1106-7.

21. Raichle ME. A brief history of human brain mapping. Trends Neurosci. 2009;32(2):118-26.

22. Gerchen MF, Kirsch P. Combining task-related activation and connectivity analysis of fMRI data reveals complex modulation of brain networks. Hum Brain Mapp. 2017;38(11):5726-39.

23. Lin CH, Chiang MC, Wu AD, Iacoboni M, Udompholkul P, Yazdanshenas O, et al. Enhanced motor learning in older adults is accompanied by increased bilateral frontal and fronto-parietal connectivity. Brain Connect. 2012;2(2):56-68.

Appendices

Summary

Throughout the lifespan, humans learn new motor skills and relearn motor skills after an injury. In older adults, motor learning is particularly important because adaptations to age-related peripheral and central neural changes are required. Increasing age is accompanied by impairments in the neuromuscular system such as sarcopenia, changes to peripheral nerve fibers, and a decrease in the number and size of motor units. In addition, deteriorations in brain structure occur, including decreases in gray and white matter volume, increases in cerebrospinal fluid volume, and decreases in regional white matter integrity. Whether or not skill acquisition and consolidation are impaired in older compared with young adults is unclear. Furthermore, it is unclear whether and how the underlying neural mechanisms of motor learning change with advancing age. A better understanding of the mechanisms of how age affects motor skill acquisition and consolidation would help design motor interventions counteracting age-related declines in motor function. In this thesis, we examined age-related differences in the underlying neural mechanisms of motor learning. In the main part of this thesis, a visuomotor tracking task was used in which participants performed wrist flexion and extension movements to follow a pre-programmed template on a computer screen. In a review paper in chapter 4, similar visuomotor tracking tasks were used with wrist and index finger movements, and additionally ballistic motor tasks were analyzed. Ballistic tasks consisted of a rapid sequential finger-to-thumb opposition task, or maximizing peak acceleration of the thumb, index finger(s), or wrist. Non-invasive neurostimulation (transcranial magnetic stimulation; TMS) and neuroimaging (functional magnetic resonance imaging; fMRI) techniques were used in this thesis to measure markers of neural plasticity after both the acquisition and motor memory consolidation phase. TMS measures excitatory and inhibitory processes of use-dependent synaptic plasticity by stimulating the primary motor cortex, whereas fMRI provides an indication of brain activation during task execution by measuring the blood-oxygen-level dependent (BOLD) signal in the whole brain.

In **chapter 2**, we examined how corticospinal excitability (CSE) and short-interval intracortical inhibition (SICI), both at rest and during the execution of the task, changed in healthy older adults after learning a visuomotor tracking task. The results indicate that a reduction in intracortical inhibition underlies motor skill acquisition and especially consolidation in healthy older adults. In contrast, CSE was not modulated at rest and during task execution. Elaborating on these findings, **chapter 3** examined age-related differences in corticospinal and intracortical excitability after visuomotor learning by comparing the data of healthy older adults obtained in chapter 2 to a group of young adults. The results showed that older adults performed worse on the visuomotor task compared with young adults, but that the absolute improvements in performance over time were similar in the two age groups. TMS results indicated that CSE measured at rest and during task execution, and SICI during task execution changed in opposite directions after motor memory consolidation in the two age groups. These results suggest that older adults use alternative strategies to consolidate a newly acquired visuomotor skill into motor memory.

In **chapter 4**, a systematic review and meta-analysis was conducted to provide an overview of 11 TMS studies regarding ballistic and visuomotor skill acquisition in aging. Additionally, this review examined for the first time the relationship between motor skill acquisition and changes in

TMS variables using individual data of 123 older and 128 young adults. Results revealed subtle age-related differences but clear task-related differences in use-dependent neural plasticity. Furthermore, increases in CSE or decreases in SICI are not related to motor skill acquisition in healthy young or older adults.

In addition to excitability changes in specific brain areas such as the primary motor cortex, motor learning requires the involvement of a wide network of brain regions including cortico-cerebellar and cortico-striatal networks. Therefore, in **chapter 5**, fMRI was applied to examine age-related differences in brain activation changes in the whole brain after visuomotor learning. Results showed age-related impairments in motor performance but similar learning rates of the visuomotor skill in young and older adults. fMRI results revealed no age-related changes over time in brain activation. However, changes in brain deactivation after consolidating the motor skill into motor memory were age-dependent, showing greater deactivations from post-test to retention in the bilateral precuneus and left occipital and temporal areas in young adults, but smaller deactivations in the left inferior frontal area in older adults. Furthermore, older adults activated cortical and subcortical areas to a greater extent than young adults during task execution. These results suggest that older adults use an alternative strategy compared with young adults while learning a visuomotor tracking skill, which might be a compensatory mechanism for age-related structural changes.

Finally, **chapter 6** provides a discussion of the results obtained in this thesis and provides recommendations for further research. To conclude, age-related differences in neural plasticity are mainly present after consolidation of a visuomotor skill but not necessarily or to a lesser extent after visuomotor skill acquisition. Because both age groups showed similar magnitudes of skill retention, these results support the compensation hypothesis. TMS measures are not related to motor skill acquisition, emphasizing the need for the adaptation of research methods or application of combined neurostimulation and neuroimaging techniques to gain better insights into age-related differences in neural plasticity. Neurorehabilitation programs including neurostimulation techniques are encouraged to optimize the stimulation protocols to the age group and stage of motor learning.

Samenvatting

Gedurende de levensduur leren mensen nieuwe motorische vaardigheden en leren ze opnieuw motorische vaardigheden uit te voeren na een blessure of aandoening. Voor oudere mensen is motorisch leren erg belangrijk omdat zij zich moeten aanpassen aan leeftijd-gerelateerde veranderingen in het perifere en centrale zenuwstelsel. Ouder worden gaat gepaard met achteruitgangen in het neuromusculaire systeem zoals het verlies van spiermassa, veranderingen in perifere zenuwvezels, en een afname in het aantal en de grootte van motorische eenheden. Daarnaast treden er achteruitgangen op in de structuur van de hersenen. Voorbeelden hiervan zijn een kleiner volume van grijze en witte stof, een groter volume aan hersenvocht, en een afname in integriteit van lokale witte stof. Het is onduidelijk of het aanleren van motorische vaardigheden (*motor skill acquisition*) en het versterken van deze vaardigheid in het motorisch geheugen (*motor memory consolidation*) achteruitgaat bij ouderen ten opzichte van jongeren. Bovendien is het onduidelijk of en hoe de onderliggende neurale mechanismen van motorisch leren veranderen tijdens veroudering. Meer kennis van de effecten van leeftijd op de mechanismen van *motor skill acquisition* en *consolidation* kan helpen om motorische interventies te ontwikkelen die leeftijd-gerelateerde achteruitgangen in motorische functies tegengaan. Dit proefschrift onderzoekt de effecten van leeftijd op de onderliggende mechanismen van motorisch leren. In het grootste gedeelte van dit proefschrift werd een visuomotorische volgtaak gebruikt waarin deelnemers buig- en strekbewegingen van de pols maakten om een voorgeprogrammeerd patroon op een computerscherm te volgen. In een overzichtsartikel in hoofdstuk 4 werden soortgelijke visuomotorische volgtaken gebruikt met pols- en wijsvingerbewegingen, en daarnaast werden ballistische motorische taken geanalyseerd. De ballistische taken bestonden uit een snelle seriële vinger-naar-duim taak, of het maximaliseren van de piek versnelling van de duim, wijsvinger(s) of pols. In dit proefschrift werden een neurostimulatie techniek (transcraniële magnetische stimulatie; TMS) en een beeldvormingstechniek (functionele magnetische resonantie beeldvorming: fMRI) gebruikt om markers van neurale plasticiteit te meten na zowel de *motor skill acquisition* als de *motor memory consolidation* fase. Neurale plasticiteit is het vermogen van de hersenen om zich aan te passen aan veranderingen. Deze veranderingen kunnen betrekking hebben op ervaringen of aandoeningen. Dit proefschrift focust zich op de neurale veranderingen na het leren van een motorische taak. TMS meet prikkelende en remmende processen van gebruiksafhankelijke synaptische plasticiteit door het gebied in de hersenen te stimuleren dat verantwoordelijk is voor het maken van vrijwillige bewegingen (de primaire motor cortex). fMRI geeft een indicatie van hersenactivatie tijdens het uitvoeren van een taak door het bloed-zuurstof-niveau afhankelijke signaal (BOLD signaal) te meten in het gehele brein.

Hoofdstuk 2 onderzoekt hoe corticospinale prikkelbaarheid (CSE) en korte-interval intracorticale inhibitie (SICI), zowel in rust als tijdens het uitvoeren van de taak, veranderen in gezonde ouderen na het leren van een visuomotorische volgtaak. De resultaten wijzen erop dat in gezonde ouderen een verlaging van intracorticale inhibitie een onderliggend mechanisme is van *skill acquisition* en vooral van *motor memory consolidation*. Voortbordurend op deze resultaten onderzoekt **hoofdstuk 3** leeftijd-gerelateerde verschillen in CSE en SICI na visuomotorisch leren door de data van de gezonde ouderen verkregen in hoofdstuk 2 te vergelijken met een groep jongeren. De resultaten toonden aan dat ouderen slechter presteren op de visuomotorische taak in vergelijking

met jongeren, maar dat de absolute verbeteringen in prestatie over de tijd gelijk is in de twee groepen. TMS-resultaten lieten zien dat CSE gemeten in rust en tijdens het uitvoeren van de taak, en SICI tijdens het uitvoeren van de taak in de twee leeftijdsgroepen in tegenovergestelde richting veranderden na *motor memory consolidation*. Deze resultaten suggereren dat ouderen alternatieve strategieën gebruiken om de nieuwgeleerde visuomotorische vaardigheid te versterken in het motorisch geheugen.

Hoofdstuk 4 bestaat uit een systematisch overzichtsartikel waarin een meta-analyse is uitgevoerd om een overzicht te geven van 11 TMS-studies die ballistische en visuomotorische *skill acquisition* tijdens veroudering onderzochten. Bovendien onderzocht dit hoofdstuk voor de eerste keer de relatie tussen *motor skill acquisition* en veranderingen in TMS-variabelen door gebruik te maken van individuele data van 123 ouderen en 128 jongeren. De resultaten lieten subtiele leeftijd-gerelateerde verschillen maar duidelijke taak-gerelateerde verschillen zien in gebruiksafhankelijke neurale plasticiteit. Verder waren verhogingen in CSE en verlagingen in SICI niet gerelateerd aan *motor skill acquisition* in beide leeftijdsgroepen.

Naast veranderingen in prikkelbaarheid van specifieke hersengebieden zoals de primaire motor cortex, vereist motorisch leren de betrokkenheid van een breed netwerk van hersengebieden inclusief de cortico-cerebellair en cortico-striatale netwerken. Daarom werd in **hoofdstuk 5** fMRI toegepast om leeftijd-gerelateerde verschillen in breinactivatie veranderingen na visuomotorisch leren te onderzoeken in het gehele brein. De resultaten toonden een leeftijd-gerelateerde verslechtering in motorische prestatie, maar gelijke leercurves van de visuomotorische vaardigheid bij jongeren en ouderen. fMRI-resultaten brachten geen leeftijd-gerelateerde verschillen aan het licht met betrekking tot veranderingen in hersenactivatie. Echter, de veranderingen in deactivering van de hersen, waarbij minder hersenactiviteit is tijdens het uitvoeren van een taak in vergelijking met rust, na de versterking van de vaardigheid in het motorisch geheugen, waren afhankelijk van leeftijd. Er waren grotere deactivaties van de na-test tot de retentie test in de bilaterale precuneus en linker occipitale en temporale gebieden in jongeren, maar kleinere deactivaties in het linker inferieure frontale gebied in ouderen. Verder toonden ouderen meer hersenactivatie tijdens het uitvoeren van de taak. Deze resultaten suggereren dat ouderen een alternatieve strategie gebruiken tijdens het leren van een visuomotorische volgvaardigheid. Dit is wellicht een compensatiemechanisme voor leeftijd-gerelateerde structurele veranderingen in de hersenen.

Tenslotte geeft **hoofdstuk 6** een discussie van de resultaten die verkregen zijn in dit proefschrift en geeft het aanbevelingen voor verder onderzoek. Concluderend, leeftijd-gerelateerde verschillen in neurale plasticiteit zijn vooral aanwezig na *consolidation* van een visuomotorische vaardigheid maar niet noodzakelijkerwijs of in kleinere mate na visuomotorische *skill acquisition*. Omdat beide leeftijdsgroepen de vaardigheid in gelijke mate behouden na 24 uur, ondersteunen deze resultaten de compensatie hypothese. TMS-maten zijn niet gerelateerd aan *motor skill acquisition*. Dit benadrukt de noodzaak voor aanpassingen van onderzoeksmethoden of de toepassing van gecombineerde neurostimulatie en beeldvormingstechnieken om meer inzicht te krijgen in de leeftijd-gerelateerde verschillen in neurale plasticiteit. Neurorevalidatie programma's die neurostimulatie technieken toepassen worden aangemoedigd om stimulatie protocollen te optimaliseren voor de leeftijdsgroep en de fase van motorisch leren.

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About the author

Kelly Berghuis was born on April 4th 1992 in Zwolle. She was raised in Raalte. She always has had a great interest in the human body and was very beta oriented. After not being selected for studying Medicine, she started to study Human Movement Sciences at the University of Groningen in 2010. Kelly received her Bachelor's degree in 2013 and her Master's degree with cum laude in 2015. During her studies, her interest in the human brain increased. She decided to apply to the ambitious MS-PhD track in which she could develop her own project. Her project focused on the age-related changes in neural plasticity after motor learning, which resulted in the current thesis. For three papers in this thesis, Kelly received a Top Publication award from the Graduate School of Medical Sciences. Additionally, she was awarded the Neural Control of Movement Scholarship award in 2018. During her PhD studies, Kelly lived for seven months in Rome, Italy, to work at the labs of prof. dr. Giacomo Koch and dr. Marco Bozzali. Here, she was given the opportunity to execute one of the experiments described in this thesis.

During her studies, Kelly participated in several teaching activities such as tutoring, guiding seminars, and being a mentor of first year students. Currently, Kelly continues her educational career by working at the Center for Human Movement Sciences in Groningen as a junior teacher.

Journal publications

Berghuis, K.M.M., Fagioli, S., Maurits, N.M., Zijdewind, I., Marsman, J.B.C., Hortobágyi, T., Koch, G., Bozzali, M., Age-related changes in brain deactivation but not in activation after motor learning, NeuroImage (2019), doi: 10.1016/j.neuroimage.2018.11.010.

Berghuis, K.M.M., Semmler, J.G., Opie, G.M., Post, A.K., Hortobágyi, T., Age-related changes in corticospinal excitability and intracortical inhibition after upper extremity motor learning: a systematic review and meta-analysis, Neurobiology of aging (2017), doi: 10.1016/j.neurobiolaging.2017.03.024.

Berghuis, K.M.M., De Rond, V., Zijdewind, I., Koch, G., Veldman, M.P., Hortobágyi, T., Neuronal mechanisms of motor learning are age-dependent, Neurobiology of aging (2016), doi: 10.1016/j.neurobiolaging.2016.06.013.

Berghuis, K.M., Veldman, M.P., Solnik, S., Koch, G., Zijdewind, I., Hortobágyi, T., Neuronal mechanisms of motor learning and motor memory consolidation in healthy old adults. Age (2015), doi: 10.1007/s11357-015-9779-8.

Négyesi, J., Veldman, M.P, **Berghuis, K.M.M.**, Javet, M., Tihanyi, J., Hortobágyi, T., Somatosensory electrical stimulation does not augment motor skill acquisition and intermanual transfer in healthy young adults – a pilot study, Motor Control (2018), doi: 10.1123/mc.2016-0048.

Veldman, M.P., Zijdewind, I., Solnik, S., Maffiuletti, N.A., **Berghuis, K.M.**, Javet, M., Negyesi, J. Hortobágyi, T., Direct and crossed effects of somatosensory electrical stimulation on motor learning and neuronal plasticity in humans, Eur. J. Appl. Physiol. (2015), doi: 10.1007/s00421-015-3248-z.

Conference contributions

Hortobágyi, T.*, **Berghuis, K.M.M.** Effects of healthy aging on motor learning and motor memory consolidation. X Congreso Internacional de la Asociación Española de Ciencias del Deporte (AECD), A Coruña, Spain, November 21-23, 2018. Oral presentation.

Berghuis, K.M.M.* Brain mechanisms of motor learning in health and disease: motor learning in aging. Neural Control of Movement 2018 meeting, Santa Fe, New Mexico, United States of America, May 1st – 4th 2018. Symposium presentation.

Berghuis, K.M.M.*, Fagioli, S., Maurits, N.M., Zijdewind, I., Marsman, J.B.C., Hortobágyi, T., Koch, G., Bozzali, M. Age-related differences in brain activity and changes in deactivation during visuomotor skill learning. Neural Control of Movement 2018 meeting, Santa Fe, New Mexico, United States of America, May 1st - 4th 2018. Poster presentation.

Berghuis, K.M.M.*, Fagioli, S., Maurits, N.M., Zijdewind, I., Marsman, J.B.C., Hortobágyi, T., Koch, G., Bozzali, M. Changes in brain activation but not deactivation during visuomotor learning are similar between older and young adults. Vereniging voor Bewegingswetenschappen Nederland (Society for Human Movement Sciences in The Netherlands) PhD day, Rotterdam, The Netherlands, December 1st 2017. Poster presentation.

Berghuis, K.M.M.*, Fagioli, S., Maurits, N.M., Zijdewind, I., Marsman, J.B.C., Hortobágyi, T., Koch, G., Bozzali, M. Age-related differences in brain activation during the acquisition and retention of a visuomotor skill. Society for Neuroscience 2017 Annual Meeting, Washington D.C., United States, November 11th – 15th 2017. Poster presentation.

Berghuis, K.M.M.*, De Rond, V., Zijdewind, I., Koch, G., Veldman, M.P., Hortobágyi, T. Neuronal mechanisms of motor learning are age-dependent. International workshop 'Aging in the neuro-musculo-skeletal system', Marseille, France, March 2016. Oral presentation.

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